Determining Absolute Configurations of Stereocenters in Annonaceous Acetogenins through Formaldehyde Acetal Derivatives and Mosher Ester Methodology

Zhe-ming Gu, Lu Zeng, Xin-ping Fang, Trina Colman-Saizarbitoria, Mei Huo, and Jerry L. McLaughlin*

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907

Received May 31, 1994®

Formaldehyde (methylene) acetal derivatives can be conveniently prepared, on a small scale, using parent Annonaceous acetogenins which have 1,2-, 1,4-, and/or 1,5-diols along their aliphatic chains. The resulting cyclic acetal protons give NMR signals which allow characterization of the relative stereochemistries of the two stereogenic centers that originated from the diols. Less complicated (vs the parent acetogenins) per-Mosher ester [methoxy(trifluoromethyl)phenyl acetate or MTPA] derivatives of the acetal derivatives can then be prepared and used to determine absolute configurations of the chiral positions which bear the remaining free hydroxyls. Prior knowledge of relative stereochemical relationships then permits assignments of absolute configurations to additional chiral centers along the chain of the molecules. This method has been particularly useful in solving the absolute configurations of several nonadjacent bis-THF and mono-THF acetogenins, *viz.* bullatanocin (**1**), (2,4-*cis* and *trans*)-bullatanocinones (**2** and **3**), bullatalicin (**4**), (2,4-*cis* and *trans*)-bullatalicinones (**5** and **6**), squamostatin A (**7**), squamocin (**8**), gigantetrocin A (**9**), and goniothalamicin (**10**). Most of the resulting acetals (vs the parent acetogenins) show enhanced bioactivities, and their mode of action is, likewise, by mitochondrial inhibition.

Introduction

The Annonaceous acetogenins are γ-lactone derivatives of C-32 or C-34 long chain fatty acids; most possess one or two tetrahydrofuran rings and a combination of double bonds, hydroxyls, ketones, epoxides, or acetoxyls as functional groups. Since their discovery in 1982, they have attracted considerable interest among natural product chemists because of their stereochemical diversities and their broad spectrum of bioactivities.[1] These compounds act biologically, at least in part, as highly potent mitochondrial inhibitors.[2] A recent review revealed that 61 acetogenins, out of a total of *ca.* 90, have been discovered in the 3 year period of 1990–92 and demonstrate the rapid growth of recent scientific information regarding this relatively new class of bioactive natural compounds.[1b]

Annonaceous acetogenins are mainly composed of three groups, *i.e.*, the adjacent bis-tetrahydrofuran, nonadjacent bis-THF, and mono-THF subclasses.[1] All of the acetogenins in these subclasses have multiple stereogenic centers, and, indeed, some are differentiated from each other only by their stereochemistries. Consequently, the determination of the relative and absolute stereochemistries of these stereocenters has become a major concern in the elucidation of the structures of new, as well as previously reported, acetogenin compounds; in addition, the stereochemistries, in many cases, influence the relative potencies and biological specificities.[1,2] Because of their waxy nature, the acetogenins and their derivatives do not readily produce crystals suitable for X-ray crystallographic analysis. Relative stereochemistries around the THF ring(s) and those of the keto lactone moieties have typically been determined by comparisons with synthetic model compounds of known relative stereochemistry.[3] The absolute stereochemistry of none of the Annonaceous acetogenins had been defined until recently when Mosher ester methodology was applied and demonstrated to be very helpful.[4] So far, the absolute configurations of the carbinol centers of several adjacent bis-THF and some mono-THF acetogenins have been determined by the use of this methodology.[4,5]

The nonadjacent bis-THF acetogenins are the newest subclass of the THF-bearing Annonaceous acetogenins,[1a,b] and some, *e.g.*, bullatalicin (**4**), show promising *in vivo* antitumor activities although their potencies are less than those of the adjacent bis-THF compounds.[2c] Their nonadjacent bis-THF rings have made their structural elucidations and assignments of their relative stereochemistries more difficult than the adjacent bis-THF and mono-THF subclasses. Bullatalicin (**4**), whose structure

® Abstract published in *Advance ACS Abstracts,* August 1, 1994.

(1) Reviews on Annonaceous acetogenins include: (a) Rupprecht, J. K.; Hui, Y.-H.; McLaughlin, J. L. *J. Nat. Prod.* **1990**, *53*, 237. (b) Fang, X.-P.; Rieser, M. J.; Gu, Z.-M.; Zhao, G.-X.; McLaughlin, J. L. *Phytochem. Anal.* **1993**, *4*, 27. (c) Cave, A.; Cortes, D.; Figadere, B.; Hocquemiller, R.; Laprevote, O.; Laurens, A.; Leboeuf, M. In *Recent Advances in Phytochemistry*; Downum, K. R., Romeo, J., Stafford, H. P., Eds.; Plenum Press: New York, 1993; Vol. 27, pp 167–202.

(2) Annonaceous acetogenins are powerful inhibitors of complex I in mitochondrial electron transport systems and, thus, rapidly deplete the energy supplies of cells. They particularly show excellent potential as pesticides and antitumor agents, especially in controlling multiple drug resistant tumors: (a) Londershausen, M.; Leicht, W.; Lieb, F.; Moeschler, H.; Weiss, H. *Pesticide Sci.* **1991**, *33*, 427. (b) Lewis, M. A.; Arnason, J. T.; Philogene, B. J. R.; Rupprecht, J. K.; McLaughlin, J. L. *Pesticide Biochem. Physiol.* **1993**, *45*, 15. (c) Ahammadsahib, K. I.; Hollingworth, R. M.; Hui, Y.-H.; McLaughlin, J. L. *Life Sci.* **1993**, *53*, 1113. (d) Landolt, J. L.; Ahammadsahib, K. I.; Hollingworth, R. M.; Barr, R.; Crane, F. L.; Buerck, N. L.; McCabe, G. P.; McLaughlin, J. L. *Chemico-Biol. Interact.*, in press. Also see Table 7.

(3) (a) Hoye, T. R.; Suhadolnik, J. C. *J. Am. Chem. Soc.* **1987**, *109*, 4402. (b) Hoye, T. R.; Zhuang, Z.-P. *J. Org. Chem.* **1988**, *53*, 5578. (c) Born, L.; Lieb, F.; Lorentzen, J. P.; Moescher, H.; Nonfon, M.; Sollner, R.; Wendisch, D. *Planta Med.* **1990**, *56*, 312. (d) Hoye, T. R.; Hanson, P. R. *J. Org. Chem.* **1991**, *56*, 5092. (e) Harmange, J.-C.; Figadere, B.; Cave, A. *Tetrahedron Lett.* **1992**, *39*, 5749.

(4) Rieser, M. J.; Hui, Y.-H.; Rupprecht, J. K.; Kozlowski, J. F.; Wood, K. V.; McLaughlin, J. L.; Hanson, P. R.; Zhuang, A.; Hoye, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 10203.

(5) Gu, Z.-M.; Fang, X.-P.; Zeng, L.; Wood, K. V.; McLaughlin, J. L. *Heterocycles* **1993**, *36*, 2221.

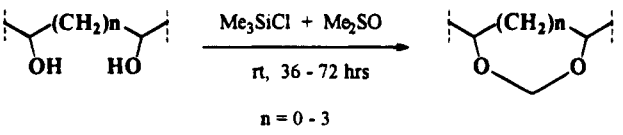
0022-3263/94/1959-5162$04.50/0

was first published in 1989, was the second member of this subclass, and its relative stereochemistry and those of others in this subclass were clarified by us in 1993.[6] The refined Mosher ester methodology analyzes differences between the proton chemical shifts of (*S*)- and (*R*)-MTPA esters on both sides of the chiral carbinol centers.[4,7] However, this procedure cannot be applied to the nonadjacent THF acetogenins, such as bullatanocin (**1**),[8] bullatalicin (**4**),[6,9] and squamostatin A (**7**),[6,10] because the hydroxyls between the two THF rings are only two carbons apart, and the phenyl rings of the Mosher esters interfere with each other; it is also not feasible to assign accurately the complicated proton chemical shifts of the per-Mosher esters of these compounds. Therefore, until now, the absolute configurations of none of the nonadjacent bis-THF acetogenins have been reported. Similar problems are also encountered in the other subclasses with acetogenins that have hydroxyls in close proximity to each other, *e.g.*, those having a vicinal diol like gigantetrocin A (**9**)[1b,11] and those having a 1,4-diol like goniothalamicin (**10**).[1a,12] In addition, the absolute configurations of non-THF-flanking carbinol centers, such as the C-28 of squamocin (**8**),[1a,13] have not been solved.[4] Consequently, many Annonaceous acetogenins remain undefined stereochemically, and many continue to be introduced into the literature without determinations of their absolute stereochemistries.

Generally, the bis-THF acetogenins show much higher cytotoxic potencies than the mono-THF compounds, and their enhanced potency is also evident in their inhibition of intact mitochondria.[1,2d] No tri-THF acetogenins have been reported, as yet, although their occurrence in nature may be predicted.[14] In addition to our desire to solve the absolute stereochemistries of the nonadjacent bis-THF acetogenins, we have been eager to determine if the bioactivities of acetogenins might be further enhanced as the number of rings is increased, *i.e.*, by conversion of the mono-THF acetogenins to bis-ring compounds and the bis-THF compounds to tri- or tetra-ring compounds.

Monoalcohols can be converted into intermolecular formaldehyde acetals using chlorotrimethylsilane (Me_3SiCl) and dimethyl sulfoxide (Me_2SO).[15] We have modified this method and successfully employed it to convert 1,2-, 1,4- and/or 1,5-diols of appropriate acetogenins into cyclic intramolecular formaldehyde acetals (Scheme 1). The acetal moiety which is formed connects the diols but

Scheme 1



does not change the stereochemistries of their carbinol centers. Significant differences in the ^1H NMR spectra[16] between the acetal protons in the *cis* or *trans* configurations of the cyclic formal derivatives then make it very easy to assign the relative stereochemistries of the diols in the parent compounds. Furthermore, the *erythro* configurations in **4**–**7** can be definitely assigned at C-23/24 and not at C-15/16 or C-19/20 after this conversion, since in their respective acetal derivatives only the THF-flanking hydroxyl at C-24 is left free and the chemical shift of H-24 at δ 3.82 clearly indicates that it is *erythro*.[3b,c]

Since this method does not affect other isolated hydroxyls, these groups, then, are free to be converted into Mosher esters. Consequently, the assignments of the proton chemical shifts affected by these Mosher esters become more feasible, *i.e.*, the formalation decreases the number of hydroxyls, decreases the number of esters, and hence, decreases the complication of proton signals affected by the Mosher esters in the esterified formal derivatives. With the relative stereochemistries around the THF ring(s) already in hand, from comparisons of ^1H NMR spectra with those of model compounds, the absolute stereochemistries of all of the stereocenters can then be concluded by analyses of the ^1H NMR data (*S*)- and (*R*)-MTPA esters of the formal derivatives.

By the use of this convenient conversion to formaldehyde acetals and the subsequent application of the refined Mosher ester methodology, we have determined the absolute stereochemistries of several Annonaceous acetogenins available in our laboratory; each represents some type of special structural feature, and all have 1,2-, 1,4-, and/or 1,5-diol moieties. Thus, bullatanocin (**1**),[1b,8] (2,4-*cis* and *trans*)-bullatanocinones (**2** and **3**),[1b,8] bullatalicin (**4**),[6,9] (2,4-*cis* and *trans*)-bullatalicinones (**5** and **6**),[6,17] squamostatin A (**7**),[6,10] squamocin (**8**),[1a,13] gigantetrocin A (**9**),[1b,11] and goniothalamicin (**10**)[1a,12] are presented as models; the results presented here define, for the first time, the absolute configurations of these compounds. It is also important to note that most of the acetal derivatives show enhanced cytotoxicities against certain human tumor cell lines; we subsequently determined that these acetal derivatives similarly show enhancements in a bioassay that quantitates the inhibition of oxygen uptake by rat liver mitochondria (RMB, Table 7),[2c,d] demonstrating that this site of their biological action is identical to that of their parent acetogenins and proving that some unanticipated mode of cytotoxic action had not been created by the addition of the acetal ring(s). Thus, enhancement of the bioactive potencies and selectivities, through preparation of these simple acetals, may permit improved utilization of many of the natural acetogenins.

(6) Fang, X.-P.; Gu, Z.-M.; Rieser, M. J.; Hui, Y.-H.; McLaughlin, J. L. *J. Nat. Prod.* **1993**, *56*, 1095.
(7) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.
(8) Gu, Z.-M.; Fang, X.-P.; Rieser, M. J.; Hui, Y.-H.; Miesbauer, L. R.; Smith, D. L.; Wood, K. V.; McLaughlin, J. L. *Tetrahedron* **1993**, *49*, 747.
(9) Hui, Y.-H.; Rupprecht, J. K.; Anderson, J. E.; Liu, Y.-M.; Smith, D. L.; Chang, C.-J.; McLaughlin, J. L. *Tetrahedron* **1989**, *45*, 6948.
(10) Fujimoto, Y.; Murasaki, C.; Kakinuma, K.; Eguchi, T.; Ikekawa, N.; Furuya, M.; Hirayama, K.; Ikekawa, T.; Sahai, M.; Gupta, Y. K.; Ray, A. B. *Tetrahedron Lett.* **1990**, *31*, 535.
(11) Fang, X.-P.; Rupprecht, J. K.; Alkofahi, A.; Hui, Y.-H.; Liu, Y.-M.; Smith, D. L.; Wood, K. V.; McLaughlin, J. L. *Heterocycles* **1991**, *32*, 11.
(12) Alkofahi, A.; Rupprecht, J. K.; Smith, D. L.; Chang, C.-J.; McLaughlin, J. L. *Experientia* **1988**, *44*, 83.
(13) Fujimoto, Y. Eguchi, T.; Kakinuma, K.; Ikekawa, N.; Sahai, M.; Gupta, Y. K. *Chem. Pharm. Bull.* **1988**, *36*, 4802.
(14) For example, if bullatanocin (**1**) or bullatalicin (**4**) were to lose one molecule of H_2O between their C-16 and C-19 hydroxyl groups, another THF ring would result, and the product would be a tri-THF acetogenin; we would predict, however, that the THF rings are formed by cyclization from epoxides rather than dehydration; see: Gu, Z.-M.; Fang, X.-P.; Zeng, L.; Song, R.; Ng, J. H.; Wood, K. V.; Smith, D. L.; McLaughlin, J. L. *J. Org. Chem.* **1994**, *59*, 3472.
(15) Bal, B. S.; Pinnick, H. W. *J. Org. Chem.* **1979**, *44*, 3727.
(16) The ^1H NMR signals of the acetal protons of *cis*-4,7-dimethyl-1,3-dioxacycloheptanes are at δ 5.47 and 5.16, and those of both acetal protons of *trans*-4,7-dimethyl-1,3-dioxacycloheptanes are at δ 5.30: Gianni, M. H.; Saavedra, J.; Savoy, J. *J. Org. Chem.* **1973**, *38*, 3971.
(17) Hui, Y.-H.; Rupprecht, J. K.; Anderson, J. E.; Wood, K. V.; McLaughlin, J. L. *Phytotherapy Res.* **1991**, *5*, 124.

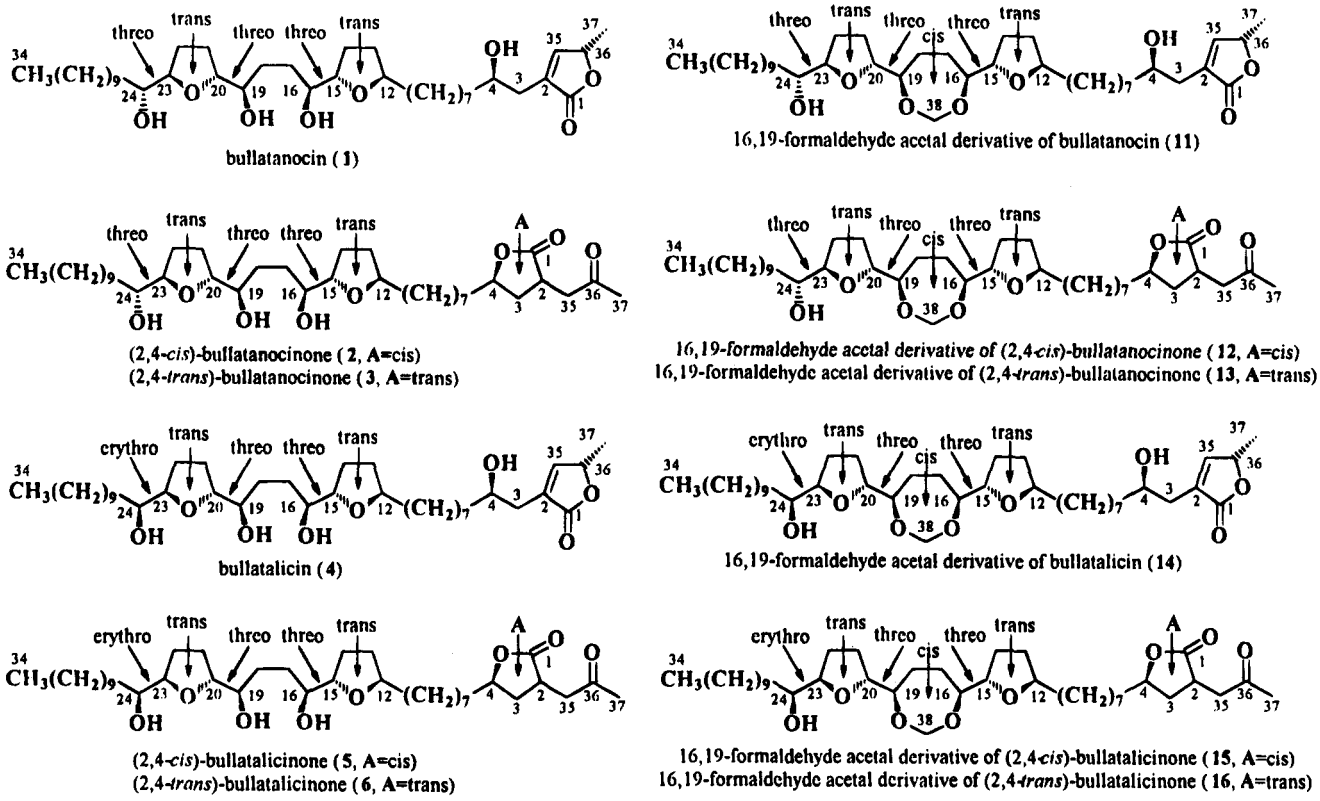


Figure 1.

Results and Discussion

As discussed above, the absolute configurations of the Annonaceous acetogenins having vicinal (1,2-), 1,4-, and/or 1,5-diols cannot be conveniently revealed by simply preparing per-Mosher ester derivatives. To determine their absolute configurations, the diols can be first converted into cyclic formaldehyde acetals; this procedure decreases the number of free hydroxyls, does not change the configuration of the carbinol centers, and indicates the relative stereochemical relationships between the hydroxylated carbon centers of the diols.

Bal *et al.*[14] mixed equivalent millimolar concentrations of monoalcohols, Me_3SiCl, and Me_2SO and converted the monoalcohols into intermolecular formaldehyde acetals. When we used this method, adding equivalent millimolar concentrations of Me_3SiCl and Me_2SO to acetogenins having 1,2- 1,4-, and/or 1,5-diols, cyclic intramolecular formaldehyde acetal derivatives of the acetogenins were obtained, but the yields were very low and did not increase by lengthening the reaction times. Thin layer chromatography (TLC) showed an abundance of unreacted acetogenins. By increasing the amount of Me_3SiCl and Me_2SO to 2–3-fold millimolar excesses over the starting acetogenins, the yields of the acetal derivatives increased to about 30% after 36–72 h reaction times. Because the reaction produces HCl, which causes decomposition of the acetogenins, further elongation of the reaction time did not increase, but, conversely, decreased the product yields. Some unreacted acetogenins can be recovered when purifying the acetal products.

(*R*)-(−)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*R*)-MTPA-Cl][18] and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*S*)-MTPA-Cl][19] are now commercially available; these and the procedure described in the Experimental Section greatly simplify the routine preparation of the Mosher esters. In conducting the analyses of the ^1H NMR data of the (*S*)- and (*R*)-MTPA esters, we have included also the chemical shift changes for the methylene protons of the THF and acetal rings; the shifts of the methylenes of the THF rings were not included in the paper of Rieser *et al.*,[4] but we observed that these data showed more distinct changes and made stronger cases for the absolute stereochemical assignments.

Bullatanocin (**1**, Figure 1) has two nonadjacent THF rings and three THF flanking hydroxyls; all of the hydroxyls possess the *threo* relative stereochemical relationships with their respective rings.[8] The ^1H NMR spectra of the (*S*)- and (*R*)-per-MTPA esters of **1** were too complicated to permit confident assignments. **1** was treated with excesses of Me_3SiCl and Me_2SO at room temperature for 48 h to give a stable product which was identified as the 16,19-formaldehyde acetal derivative (**11**, yield: 30%). The structure of **11** (Figure 1) was determined by the ^1H and ^{13}C NMR data and confirmed by the HRFABMS [obsd 651.4828, calcd 651.4836, for $C_{38}H_{67}O_8$ (MH^+)] and the EIMS fragmentation of its bis-TMSi derivative. The downfield shifts of two hydroxy methine (H-16 and 19) protons from δ 3.41 in **1**[8] to δ 3.66 and 3.63 in **11** and the appearance of two doublets (J = 7.5 Hz) at δ 5.26 and 4.63 (the acetal protons), in the ^1H NMR spectrum of **11** (Table 1), and a carbon signal at δ 95.8 (the acetal carbon), in the ^{13}C NMR spectrum of **11**, confirmed the formation of an acetal ring in **11**. These NMR data also indicated that the newly formed acetal ring possessed the *cis* relative configuration[16] and, thus, revealed either an *S*/*R* or an *R*/*S* relative configuration between C-16 and C-19. Two hydroxylated methine protons at δ 3.84 and 3.39 in the ^1H NMR spectra of **11**

(18) Aldrich catalog no. 36,966-7.
(19) Aldrich catalog no. 36,967-5.

Table 1. ^{1}H NMR Data of 11, 14, (*S*)- and (*R*)-Per-MTPA-11, and (*S*)- and (*R*)-Per-MTPA-14 [δ ppm (J = Hz)]

proton	11	(*S*)-11	(*R*)-11	$\Delta\delta_H(\delta_S - \delta_R)$	14	(*S*)-14	(*R*)-14	$\Delta\delta_H(\delta_S - \delta_R)$
37	1.44 d (7.0)	1.29 d (7.0)	1.32 d (7.0)	neg	1.43 d (7.0)	1.29 d (7.0)	1.32 d (7.0)	neg
36	5.06 qq	4.86 qq	4.91 qq	neg	5.07 qq	4.86 qq	4.91 qq	neg
35	7.19 q (1.5)	6.72 q (1.5)	6.98 q (1.5)	neg	7.19 q (1.5)	6.72 q (1.5)	6.98 q (1.5)	neg
3a	2.53 ddtd	2.60	2.68	neg	2.53 ddtd	2.60	2.68	neg
3b	2.40 ddt	2.58	2.60	neg	2.40 ddt	2.58	2.60	neg
4	3.84 m	5.31 m	5.37 m	*R*[a]	3.84 m	5.31 m	5.37 m	*R*[a]
5	1.47 m	1.64 m	1.62 m	pos	1.47 m	1.65 m	1.62 m	pos
6–10	1.40–1.20	1.40–1.20	1.40–1.20		1.40–1.20	1.40–1.20	1.40–1.20	
11a	1.47 m	1.47 m	1.47 m		1.48	1.48	1.48	
11b	1.40 m	1.38 m	1.38 m		1.42 m	1.40 m	1.38 m	
12	3.95 m	3.94 m	3.94 m		3.96 m	3.94 m	3.94 m	
13a	2.01 m	2.01 m	2.01 m		2.00 m	2.02 m	2.02 m	
13b	1.64 m	1.62 m	1.61 m		1.64 m	1.64 m	1.63 m	
14a	1.96 m	1.93 m	1.93 m		2.00 m	1.94 m	1.94 m	
14b	1.66 m	1.62 m	1.61 m		1.63 m	1.64 m	1.64 m	
15	3.99 m	3.98 m	3.99 m		4.06 m	4.01 m	4.00 m	
16	3.66 m	3.59 m	3.63 m	neg	3.62 m	3.62 m	3.62 m	
17a, 18a	1.87 m	1.82 m	1.92 m	neg	1.87 m	1.88 m	1.84 m	pos
17b, 18b	1.79 m	1.70 m	1.76 m	neg	1.79 m	1.76 m	1.72 m	pos
38a	5.26 d (7.5)	5.21 d (7.5)	5.22 d (7.5)	neg	5.29 d (7.5)	5.28 d (7.5)	5.23 d (7.5)	pos
38b	4.63 b (7.5)	4.56 d (7.5)	4.56 d (7.5)	~0	4.64 d (7.5)	4.62 d (7.5)	4.56 d (7.5)	pos
19	3.63 m	3.61 m	3.63 m	neg	3.62 m	3.62 m	3.54 m	pos
20	4.01 m	3.94 m	4.02 m	neg	4.00 m	3.95 m	3.74 m	pos
21a	1.96 m	1.75 m	1.93 m	neg	2.00 m	1.90 m	1.82 m	pos
21b	1.66 m	1.63 m	1.77 m	neg	1.63 m	1.67 m	1.60 m	pos
22a	1.97 m	1.91 m	2.03 m	neg	1.90 m	1.93 m	1.85 m	pos
22b	1.64 m	1.51 m	1.59 m	neg	1.83 m	1.79 m	1.70 m	pos
23	3.84 m	4.08 m	4.08 m	~0	3.94 m	4.13 m	4.05 m	pos
24	3.39 m	5.06 m	5.05 m	*R*[a]	3.88 m	5.33 m	5.29 m	*S*[a]
25	1.40 m	1.62 m	1.47 m	pos	1.35 m	16.0 m	1.62 m	neg
26	1.40–1.20	1.30 m	1.14 m	pos	1.40–1.20	1.20 m	1.30 m	neg
27~33	1.40~1.20	1.40~1.20	1.40–1.15		1.40–1.20	1.40–1.20	1.40–1.20	
34	0.878 t (7.0)	0.882 t (7.0)	0.883 t (7.0)		0.878 t (7.0)	0.883 t (7.0)	0.882 t (7.0)	
MeO–4		3.52 s	3.50 s			3.52 s	3.50 s	
MeO–24		3.55 s	3.64 s			3.54 s	3.56 s	
Ar–10*H*		7.63–7.37	7.65–7.36			7.63–7.37	7.63–7.36	

[a] Absolute configuration of carbinol center.

shifted downfield to δ 5.31 and 5.06 in (*S*)-MTPA-**11** and to δ 5.37 and 5.05 in (*R*)-MTPA-**11** and further demonstrated that only two hydroxyls, at C-4 and C-24, remained free in **11**. The assignments of the proton chemical shifts of the Mosher esters of **11** were relatively easy, because the phenyl rings were separated by 19 carbons, too far apart to interfere with each other. The ^{1}H NMR data for the bis-(*S*)- and -(*R*)-MTPA-**11** are summarized in Table 1. On the basis of Mosher's arguments,[4] C-24 was assigned to have the *R* absolute configuration, since the sign of $\Delta\delta_H$ ($\delta_S - \delta_R$) is positive for the chain side, showing relatively more shielding for this side in (*R*)-MTPA-**11**, and negative for the THF ring side, showing relatively more shielding for this side in (*S*)-MTPA-**11**. As the relative stereochemistry from C-12 to C-24 of **1** was already known,[8] the absolute configurations of C-12 (*R*), C-15 (*S*), C-16 (*S*), C-19 (*R*), C-20 (*R*), and C-23 (*R*) were readily concluded. The configuration at C-4 was determined to be *R*, according to the data listed in Table 1, and C-36 was assigned as *S*, based on the usual ubiquitous 4*R*,36*S* relationship.[4] Thus, the absolute configuration of bullatanocin (**1**) is proposed as illustrated in Figure 1.

(2,4-*cis* and *trans*)-Bullatanocinones (**2** and **3**, Figure 1)[8] are a pair of keto lactone acetogenins differing from each other only in the configuration of the 2,4-bisubstituted γ-lactone ring. Because of the close similarity of their structures, such 2,4-*cis* and *trans* keto lactone acetogenins are very difficult to separate, and they are usually isolated and reported as a pair of isomers.[1a,b] The structures of **2** and **3** are very similar to that of **1** except that they possess the keto lactone moiety instead of the α,β-unsaturated γ-lactone ring. Thus, the skeletons and relative stereochemistries around the nonadjacent bis-THF rings of **2** and **3** are the same as those of **1**, **2**, and **3**, as a mixture, were converted into the formaldehyde acetal derivatives [**12** and **13**, yield: 30%, HRFABMS: obsd 651.4836, calcd 651.4836, for $C_{38}H_{67}O_8$ (MH^+)]. The *cis*-configuration of the acetal ring was indicated by the two doublets (J = 7.5 Hz) for the acetal protons, present at δ 5.27 and 4.63, in the ^{1}H NMR spectra (Table 2) of **12** and **13**. C-24 was determined to have the *R* configuration from comparisons of the ^{1}H NMR data of (*S*)- and (*R*)-MTPA esters of **12** and **13**. These data suggested that **2** and **3**, as expected, have the same absolute configurations in the nonadjacent bis-THF moiety as those in **1**. The (2,4-*cis* and *trans*)-keto lactone acetogenins can be chemically prepared from the original 4-hydroxylated α,β-unsaturated γ-lactone acetogenins.[20] In the conversion, the configuration at C-4 will not be changed. Because the *R* configuration has been found to be universal in all of the natural 4-hydroxylated acetogenins whose absolute stereochemistries have been revealed,[6] the absolute configurations of C-4 in **2** and **3** were, thus, also assigned as 4*R*. Thus, the absolute configuration of 2,4-*cis*-bullatanocinone (**2**) is proposed to be 2*R*,4*R*,12*R*,15*S*,16*S*,19*R*,20*R*,23*R*,24*R* and that of 2,4-*trans*-bullatanocinone (**3**) is proposed to be 2*S*,4*R*,-12*R*,15*S*,16*S*,19*R*,20*R*,23*R*,24*R*.

Since there was only one free hydroxyl left in **12** and **13**, in the ^{1}H NMR spectra of (*S*)- and (*R*)-MTPA esters

(20) Hui, Y.-H.; Rupprecht, J. K.; Anderson, J. E.; Liu, Y.-M.; Smith, D. L.; Chang, C.-J.; McLaughlin, J. L. *J. Nat. Prod.* **1989**, *45*, 6941.

Table 2. ^{1}H NMR Data of 12 and 13, 15 and 16, (*S*)- and (*R*)-MTPA-12 and -13, and (*S*)- and (*R*)-MTPA-15 and −16 [δ ppm (J = Hz)]

proton	12 and 13	(*S*)-12 and -13	(*R*)-12 and -13	$\Delta\delta_H(\delta_S - \delta_R)$	15 and 16	(*S*)-15 and -16	(*R*)-15 and -16	$\Delta\delta_H(\delta_S - \delta_R)$
2 *cis*	3.02 m	3.02 m	3.02 m		3.02 m	3.02 m	3.02 m	
trans	3.03 m	3.03 m	3.03 m		3.03 m	3.03 m	3.03 m	
3a *cis*	1.48 m	1.48 m	1.48 m		1.48 m	1.48 m	1.48 m	
trans	2.23 dddd	2.23 dddd	2.23 dddd		2.23 dddd	2.23 dddd	2.23 dddd	
3b *cis*	2.61 dddd	2.61 dddd	2.61 dddd		2.61 dddd	2.61 dddd	2.61 dddd	
trans	1.99 m	1.99 m	1.99 m		1.99 m	1.99 m	1.99 m	
4 *cis*	4.39 dddd	4.39 dddd	4.39 dddd		4.39 dddd	4.39 dddd	4.39 dddd	
trans	4.54 dddd	4.54 dddd	4.54 dddd		4.54 dddd	4.54 dddd	4.54 dddd	
5a *cis*	1.76 m	1.76 m	1.76 m		1.76 m	1.76 m	1.76 m	
trans	1.71 m	1.71 m	1.71 m		1.71 m	1.71 m	1.71 m	
5b *cis*	1.60 m	1.60 m	1.60 m		1.60 m	1.60 m	1.60 m	
trans	1.58 m	1.58 m	1.58 m		1.58 m	1.58 m	1.58 m	
6–10	1.40–1.20	1.40–1.20	1.40–1.20		1.40–1.20	1.40–1.20	1.40–1.20	
11a	1.47 m	1.47 m	1.47 m		1.48 m	1.48 m	1.48 m	
11b	1.40 m	1.38 m	1.38 m		1.42 m	1.40 m	1.38 m	
12	3.95 m	3.94 m	3.94 m		3.94 m	3.95 m	3.95 m	
13a	2.01 m	2.00 m	2.00 m		2.00 m	2.02 m	2.02 m	
13b	1.64 m	1.62 m	1.61 m		1.64 m	1.64 m	1.63 m	
14a	1.96 m	1.93 m	1.93 m		2.00 m	1.95 m	1.94 m	
14b	1.66 m	1.62 m	1.62 m		1.63 m	1.65 m	1.64 m	
15	4.00 m	3.98 m	4.01 m	neg	4.04 m	4.03 m	4.00 m	pos
16	3.67 m	3.59 m	3.63 m	neg	3.62 m	3.63 m	3.61 m	pos
17a, 18a	1.87 m	1.82 m	1.92 m	neg	1.87 m	1.86 m	1.83 m	pos
17b, 18b	1.79 m	1.73 m	1.78 m	neg	1.79 m	1.78 m	1.71 m	pos
38a	5.27 d (7.5)	5.21 d (7.5)	5.22 d (7.5)	neg	5.29 d (7.5)	5.26 d (7.5)	5.23 d (7.5)	pos
38b	4.63 d (7.5)	4.56 d (7.5)	4.56 d (7.5)	~0	4.64 d (7.5)	4.59 d (7.5)	4.57 d (7.5)	pos
19	3.63 m	3.58 m	3.63 m	neg	3.62 m	3.61 m	3.54 m	pos
20	4.01 m	3.93 m	4.01 m	neg	4.01 m	3.95 m	3.74 m	pos
21a	1.96 m	1.76 m	1.93 m	neg	2.00 m	1.88 m	1.80 m	pos
21b	1.66 m	1.62 m	1.77 m	neg	1.63 m	1.66 m	1.61 m	pos
22a	1.97 m	1.92 m	2.04 m	neg	1.90 m	1.92 m	1.83 m	pos
22b	1.64 m	1.52 m	1.57 m	neg	1.83 m	1.78 m	1.70 m	pos
23	3.84 m	4.08 m	4.08 m	~0	3.94 m	4.13 m	4.05 m	pos
24	3.39 m	5.06 m	5.05 m	*R*[a]	3.88 m	5.33 m	5.28 m	*S*[a]
25	1.40 m	1.63 m	1.47 m	pos	1.35 m	1.57 m	1.62 m	neg
26	1.30–1.20	1.30 m	1.14 m	pos	1.30–1.20	1.20 m	1.30 m	neg
27–33	1.40–1.20	1.40–1.20	1.40–1.20		1.40–1.20	1.40–1.20	1.40–1.20	
34	0.878 t (7.0)	0.881 t (7.0)	0.883 t (7.0)		0.878 t (7.0)	0.883 t (7.0)	0.881 t (7.0)	
35a *cis*	2.61 dd	2.61 dd	2.61 dd		2.61 dd	2.61 dd	2.61 dd	
trans	2.67 dd	2.67 dd	2.67 dd		2.67 dd	2.67 dd	2.67 dd	
35b *cis*	3.11 dd	3.11 dd	3.11 dd		3.11 dd	3.11 dd	3.11 dd	
trans	3.04 dd	3.04 dd	3.04 dd		3.04 dd	3.04 dd	3.04 dd	
37	2.20 s	2.20 s	2.20 s		2.20 s	2.20 s	2.20 s	
MeO–24		3.56 s	3.64 s			3.54 s	3.56 s	
Ar–5H		7.63–7.37	7.65–7.36			7.63–7.37	7.65–7.36	

[a] Absolute configuration of carbinol center.

of **12** and **13**, the shielding distance that a single Mosher ester could affect was easily observed. The data listed in Table 2 show that the shielding effect of the phenyl ring could reach at least eight bonds from the chiral carbon center (C-24). This observation can be very useful in determining the absolute stereochemistries of isolated chain carbinol centers because, in those situations, it is often impossible to differentiate and make chemical shift assignments of the methylene protons closest to the carbinol center. However, the small, but very stable and reproducible, chemical shift differences of the protons on the oxygenated carbons or the terminal methyl group, located several bonds from the carbinol center, can be clearly observed in the ^{1}H NMR spectra of the respective (*S*)- and (*R*)-MTPA derivatives.[21]

Bullatalicin (**4**, Figure 1) has the same skeleton of contiguous atoms as **1**. However, **4** possesses two hydroxyls having *threo* and one hydroxyl having *erythro* relative stereochemical relationships with the THF rings; in **1** all three of the hydroxyls are *threo* to their respective THF rings. When **4** was first published, the *erythro* relationship was erroneously assigned at C-15/16.[9] This assignment was subsequently found to be incorrect by careful study of the COSY spectrum of **4**, and the location of the *erythro* linkage was revised to be at C-23/24 by the use of double relayed COSY spectra and by comparisons with the structure of other acetogenins from the same source.[6] Since the proton chemical shifts of H-16 and H-19 of **4** were very close to each other and the off-diagonal crosspeaks between H-16 and H-19 in the double relayed COSY spectrum of **4** were weak, we wished to verify this. After **4** was converted into the 16,19-formaldehyde acetal derivative (**14**, Figure 1), using Me_3SiCl and Me_2SO, both proton signals at δ 3.41 in **4**, which are assigned to the protons on the hydroxylated carbons having *threo* relationships with the THF rings, shifted downfield to δ 3.62 in **14**, and the proton signal at δ 3.88 in **4**, which is assigned to the proton on the hydroxylated carbon having an *erythro* relationship with its THF ring, remained almost unchanged in **14** (Table 1) vs **4**.[6] Thus,

(21) To illustrate this point further, the absolute stereochemistry of isoannonacin[1a] at C-10 was easily concluded to be *R* by observing such differences of the signals for H-4 in the ^{1}H NMR spectra of its per-(*S*)- and -(*R*)-MTPA esters, since annonacin is chemically convertable into isoannonacin, this procedure now defines the C-10 of annonacin and isoannonacin as *R*; the stereochemistry at C-10 of these compounds had not been previously determined.[4]

the fact that a free hydroxyl having an *erythro* relationship with its THF ring remained at C-24 in **14** confirmed the placement of the *erythro* linkage at C-23/24 in **4**. Again, the acetal protons in **14**, appearing as two doublets at δ 5.29 and 4.64, respectively, suggested an *R/S* or an *S/R* relative stereochemical relationship between C-16 and C-19. Bis (*S*)- and -(*R*)-MTPA esters of **14** were then prepared, and their ^{1}H NMR data are reported in Table 1. The absolute configurations of C-4 and C-24 were easily determined to be *R* and *S*, respectively, by the striking change of the signs of the $\Delta\delta_H(\delta_S - \delta_R)$ values on the two sides of the chiral centers (Table 1). The conclusion here clearly demonstrates that **4** differs from **1** only in the absolute stereochemistry at C-24 (*S* in **4** and *R* in **1**). The structure of **4**, showing the proposed absolute configurations (4*R*,12*R*,15*S*,16*S*,-19*R*,20*R*,23*R*,24*S*,36*S*), is illustrated in Figure 1.

(2,4-*cis* and *trans*)-Bullatalicinones (**5** and **6**, Figure 1) are another pair of keto lactone acetogenins, isolated and reported as a mixture of the two isomers.[1] As with the relationship between bullatanocin (**1**) and its keto lactones (**2** and **3**), **5** and **6** possess the same skeletons and relative stereochemistries around the nonadjacent bis-THF rings as those of bullatalicin (**4**). In the ^{1}H NMR spectrum of their 16,19-formaldehyde acetal derivatives (**15** and **16**, Figure 1), the acetal proton signals presented two doublets at δ 5.29 and 4.64 (J = 7.5) and suggested the *cis* configuration of the newly formed acetal ring.[16] The chemical shift of H-24, the only proton left on a hydroxylated carbon in **15** and **16**, appeared at δ 3.88, the same as in **14**, and this observation placed the *erythro* linkage at C-23/24 in **5** and **6**. Consequently, the (*S*)- and (*R*)-MTPA esters of **15** and **16** were prepared, and the ^{1}H NMR and COSY spectra of these esters were recorded. These data (Table 2) further demonstrated that the influence of a mono-MTPA, as in the (*S*)- and (*R*)-MTPA esters of **12** and **13**, could extend over at least eight bonds. The $\Delta\delta_H(\delta_S - \delta_R)$ values (Table 2) determined the *S* absolute configuration at C-24. Thus, as illustrated in Figure 1, the absolute configuration of 2,4-*cis*-bullatalicinone (**5**) was concluded to be 2*R*,4*R*,12*R*,15*S*,16*S*,19*R*,20*R*,23*R*,24*S* and that of 2,4-*trans*-bullatalicinone (**6**) was concluded to be 2*S*,4*R*,12*R*,15*S*,16*S*,19*R*,20*R*,23*R*,24*S*.

Squamostatin A (**7**, Figure 2) was originally published without determination of any stereochemistry.[1a] Its relative stereochemistry was assigned by us recently[6] and showed that the relative configuration around the nonadjacent bis-THF rings was exactly the same as that of bullatalicin (**4**), but its absolute stereochemistry remained unknown. The C-28 hydroxyl made the direct determination of its absolute configuration more complicated because the acetal reaction can convert both 1,4- and 1,5-diols into acetal rings and complete conversion would leave no free hydroxyl available in **7** for making Mosher esters to determine the absolute stereochemistry. We then predicted that the formation of a seven-membered acetal ring from a 1,4-diol should be faster than the formation of an eight-membered acetal ring from a 1,5-diol. The conversion of **7** to the formaldehyde acetal derivatives was carefully performed, and the progress was checked by TLC every 4 h. The reaction was terminated after 36 h when almost all of the starting material had disappeared and two major products had appeared; the two products were purified by HPLC. The ^{1}H NMR data of the two products (Table 3) indicated, just as predicted, that one product was the 16,19-

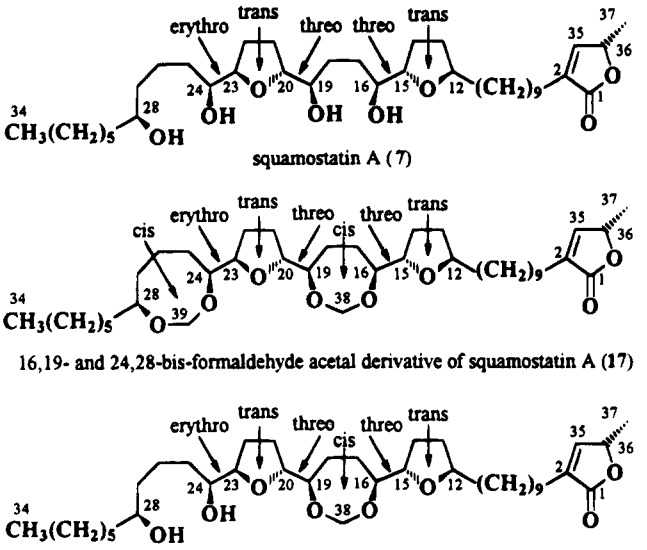


Figure 2.

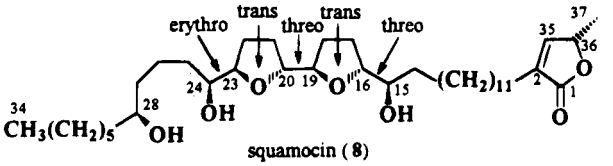
monoformaldehyde acetal derivative (**18**, Figure 2) of **7** and the other, which was much less polar than **18** on TLC, was the 16,19- and 24,28-bis-formaldehyde acetal derivative (**17**, Figure 2) of **7**. The two pairs of doublets at δ 5.27/4.62 and 5.10/4.56 in the ^{1}H NMR spectrum (Table 3) of **17** suggested that both formaldehyde acetal rings possessed the *cis* relative configuration and indicated either an *R/R* or an *S/S* relative relationship between C-24 and C-28 and either an *R/S* or an *S/R* relative relationship between C-16 and C-19 in **7**; the latter indication was confirmed by the ^{1}H NMR spectrum of **18**, in which the acetal protons also appeared as two doublets at δ 5.29 and 4.64 and were almost the same signals as those of **14**. Table 3 lists the ^{1}H NMR data of the (*S*)- and (*R*)-MTPA esters of **18** and the $\Delta\delta_H(\delta_S - \delta_R)$ values. Although the $\Delta\delta_H(\delta_S - \delta_R)$ of H-25 gave a zero value as it was adversely affected by the Mosher ester at C-28, the $\Delta\delta_H(\delta_S - \delta_R)$ values of the protons on the THF ring side of the chiral center at C-24, as far away as to H-16, showed universally positive values. This permitted us to conclude that the absolute configuration at C-24 is *S* and that at C-28 is also *S*. As noted above, in conducting the analyses of the ^{1}H NMR data of the (*S*)- and (*R*)-MTPA esters of **12** and **13** and the (*S*)- and (*R*)-MTPA esters of **15** and **16**, we had observed that the shielding influence of an MTPA moiety could reach at least eight bonds. It was, furthermore, very interesting to find that the $\Delta\delta_H(\delta_S - \delta_R)$ values of the terminal methyls (C-34) of the (*S*)- and (*R*)-MTPA esters of **18** were −7.5 Hz (Table 3) and were affected sufficiently for the application of Mosher's methodology; this result similarity suggested an *S* absolute configuration for C-28.

Squamocin (**8**, Figure 3) is an adjacent bis-THF acetogenin which has, so far, been reported from four Annonaceous plants.[1a,b] Its relative stereochemistry was determined by an X-ray crystallographic study of a derivative, but its absolute stereochemistry was not solved, since the absolute configuration at C-28 could not be determined. To solve this problem, the hydroxyls at C-24 and C-28 were converted into the formaldehyde acetal (**19**) whose ^{1}H NMR data (Table 4) showed the same relative stereochemical relationship (*R/R* or *S/S*) between C-24 and C-28 as that previously revealed by X-ray diffraction. This result also demonstrated that the

Table 3. ^{1}H NMR Data of 17, 18, and (*S*)- and (*R*)-Per-MTPA-18 [δ ppm (J = Hz)]

proton	17	18	(*S*)-18	(*R*)-18	$\Delta\delta_H(\delta_S - \delta_R)$
37	1.41 d (7.0)	1.41 d (7.0	1.41 d (7.0	1.41 d (7.0	
36	5.00 qq	5.00 qq	5.00 qq	5.00 qq	
35	6.99 q	6.99 q	6.93 q	6.93 q	
3	2.26 tt	2.26 tt	2.26 tt	2.26 tt	
4–10	1.40–1.20	1.40–1.20	1.40–1.20	1.40–1.20	
11	1.50 m	1.50 m	1.50 m	1.50 m	
12	3.94 m	3.94 m	3.95 m	3.95 m	
13a	2.02 m	2.02 m	2.03 m	2.02 m	
13b	1.48 m	1.47 m	1.49 m	1.48 m	
14a	1.98 m	2.01 m	1.95 m	1.95 m	
14b	1.64 m	1.64 m	1.64 m	1.62 m	
15	4.03 m	4.04 m	4.01 m	4.00 m	
16	3.62 m	3.62 m	3.63 m	3.60 m	pos
17a, 18a	1.82 m	1.82 m	1.86 m	1.82 m	pos
17b, 18b	1.68 m	1.76 m	1.77 m	1.72 m	pos
38a	5.27 d (7.5)	5.29 d (7.5)	5.28 d (7.5)	5.23 d (7.5)	pos
38b	4.62 d (7.5)	4.64 d (7.5)	4.61 d (7.5)	4.56 d (7.5)	pos
19	3.62 m	3.62 m	3.61 m	3.53 m	pos
20	4.00 m	4.01 m	3.93 m	3.73 m	pos
21a	1.98 m	2.01 m	1.89 m	1.82 m	pos
21b	1.64 m	1.65 m	1.66 m	1.60 m	pos
22a	1.98 m	1.90 m	1.90 m	1.81 m	pos
22b	1.92 m	1.83 m	1.77 m	1.66 m	pos
23	3.92 m	3.92 m	4.08 m	3.95 m	pos
24	3.70 m	3.89 m	5.27 m	5.19 m	*S*[a]
25	1.63, 1.43	1.48, 1.40	1.57 m	1.57 m	~0
26	1.40–1.20	1.40–1.20	1.40–1.20	1.40–1.20	
27	1.40 m	1.47 m	1.57 m	1.51 m	pos
28	3.54 m	3.60 m	5.02 m	5.03 m	*S*[a]
29	1.22 m	1.42 m	1.57 m	1.60 m	neg
30–33	1.40–1.20	1.40–1.20	1.40–1.20	1.40–1.20	
34	0.877 t (7.0)	0.883 t (7.0)	0.862 t (7.0)	0.877 t (7.0)	neg
39a	5.10 d (7.5)				
39b	4.56 d (7.5)				
MeO-24			3.54 s	3.54 s	
MeO-28			3.53 s	3.52 s	
Ar-10H			7.60–7.37	7.60–7.37	

[a] Absolute configuration of carbinol center.



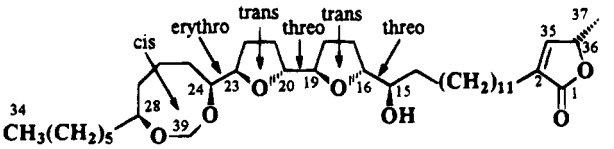


Figure 3.

relative stereochemistry of such diols could be accurately predicted by their acetal derivatives. After the absolute configuration at C-15 was determined to be *S* by analyses of the ^{1}H NMR data of the (*S*)- and (*R*)-MTPA esters of **19**, these absolute stereochemistries of the other chiral centers, around the bis-THF rings and at C-28, were easily determined by tracing their relative stereochemistries. Squamocin (**8**) has the same skeleton and relative configurations around the adjacent bis-THF rings as those of bullatacin and bullatacinone,[1a] and the above results showed that **8** also possesses the same absolute configuration within this moiety.[4] At C-28, **8** was concluded to be *S*, and by checking the $\Delta\delta_H(\delta_S - \delta_R)$ value of the terminal methyl (C-34) of the (*S*)- and (*R*(-per-MTPA esters of **8**, a negative value similarity suggested the *S* absolute configuration at C-28. This latter observation, as mentioned above in the discussion of squamostatin A (**7**), will be helpful to the stereochemical elucidations of several new acetogenins having a chain hydroxyl close to the terminal methyl (at positions C-28 to C-32).[1b,22] In such cases, it is not feasible to assign the chemical shifts of the methylene protons that are close to the chain carbinol centers in the ^{1}H NMR spectra of the Mosher esters of these compounds, but the absolute configuration of such chain carbinol centers can be easily solved by simply observing the $\Delta\delta_H(\delta_S - \delta_R)$ values of the terminal methyls.

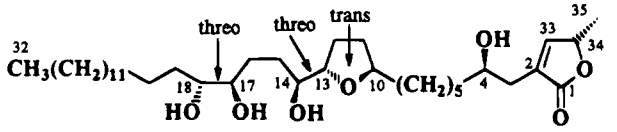
Gigantetrocin A (**9**, Figure 4) is a mono-THF acetogenin having a 1,4,5-triol moiety, and the analyses of the ^{1}H NMR spectra of the (*S*)- and (*R*)-per-MTPA esters of **9** failed to solve the absolute configuration of **9**. In addition, the triol group was anticipated to produce a mixture of formaldehyde acetal derivatives which would complicate the determination of the absolute configurations of the carbinol centers. Nevertheless, **9** was treated with excesses of Me_3SiCl and Me_2SO for 36 h. Following the routine procedures, two products were purified by HPLC. Just as predicted, the major product (yield: 35%) was the 17,18-formaldehyde acetal derivative (**21**) of **9**, and the minor product (yield: 4%) was the 14,17-formaldehyde acetal derivative (**20**) of **9**, demonstrating that it is much easier to form a five-membered acetal ring

(22) (a) Gu, Z.-M.; Fang, X.-P.; Hui, Y.-H.; McLaughlin, J. L. *Nat. Toxins* **1994**, *2*, 49. (b) Zhao, G.-X.; Ng, J. H.; Kozlowzki, J. F.; Smith, D. L. *McLaughlin*, J. L. *Heterocycles*, in press. (c) Zhao, G.-X.; Miesbauer, L. R.; Smith, D. L.; McLaughlin, J. L. *J. Med. Chem.* **1994**, *37*, 1971.

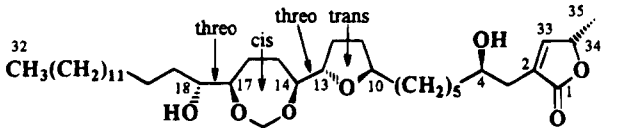
Table 4. ^{1}H NMR Data of 19, (*S*)- and (*R*)-Per-MTPA-8, and (*S*)- and (*R*)-MTPA-19 [δ ppm (J = Hz)]

proton	(*S*)-**8**	(*R*)-**8**	$\Delta\delta_H(\delta_S - \delta_R)$	**19**	(*S*)-**19**	(*R*)-**19**	$\Delta\delta_H(\delta_S - \delta_R)$
37	1.41 d (7.0)	1.41 d (7.0)		1.41 d (7.0)	1.41 d (7.0)	1.41 d (7.0)	
36	5.00 qq	5.00 qq		5.00 qq	5.00 qq	5.00 qq	
35	6.93 q	6.93 q		6.93 q	6.93 q	6.93 q	
3	2.26 tt	2.26 tt		2.26 tt	2.26 tt	2.26 tt	
4–12	1.40–1.20	1.40–1.20		1.40–1.20	1.40–1.20	1.40–1.20	
13	1.30 m	1.13 m	pos	1.40–1.20	1.33 m	1.14 m	pos
14	1.61 m	1.46 m	pos	1.39 m	1.63 m	1.50 m	pos
15	5.06 m	5.03 m	*R*[a]	3.39 m	5.06 m	5.05 m	*R*[a]
16	4.03 m	3.99 m	pos	3.82 m	4.05 m	4.045 m	~0
17a	1.92 m	2.01 m	neg	1.95 m	1.89 m	2.02 m	neg
17b	1.52 m	1.55 m	neg	1.62 m	1.50 m	1.57 m	neg
18a	1.80 m	1.87 m	neg	1.95 m	1.72 m	1.92 m	neg
18b	1.65 m	1.74 m	neg	1.62 m	1.61 m	1.70 m	neg
19	3.79 m	3.82 m	neg	3.85 m	3.79 m	3.89 m	neg
20	3.79 m	3.63 m	pos	3.90 m	3.84 m	3.89 m	neg
21a	1.80 m	1.68 m	pos	1.95 m	1.87 m	1.92 m	neg
21b	1.65 m	1.56 m	pos	1.60 m	1.59 m	1.70 m	neg
22a	1.82 m	1.75 m	pos	1.95 m	1.87 m	1.91 m	neg
22b	1.68 m	1.57 m	pos	1.80 m	1.71 m	1.73 m	neg
23	3.96 m	3.87 m	pos	3.91 m	3.80 m	3.82 m	neg
24	5.21 m	5.14 m	*S*[a]	3.68 m	3.61 m	3.61 m	
25	1.55 m	1.55 m	~0	1.64, 1.44	1.40 m	1.42 m	
26	1.40–1.20	1.40–1.20		1.40–1.20	1.40–1.20	1.40–1.20	
27	1.58 m	1.50 m	pos	1.62 m	1.62 m	1.60 m	
28	4.99 m	5.03 m	*S*[a]	3.55 m	3.54 m	3.55 m	
29	1.47 m	1.53 m	neg	1.62 m	1.57 m	1.57 m	
30–33	1.40–1.20	1.40–1.20		1.40–1.20	1.40–1.20	1.40–1.20	
34	0.860 t (7.0)	0.875 t (7.0)	neg	0.878 t (7.0)	0.877 t (7.0)	0.878 t (7.0)	
38a				5.10 d (7.5)	5.08 d (7.5)	5.09 d (7.5)	
38b				4.56 d (7.5)	5.53 d (7.5)	5.53 d (7.5)	
MeO-15					3.56 s	3.64 s	
MeO-28							
Ar-H					7.65–7.35	7.65–7.35	

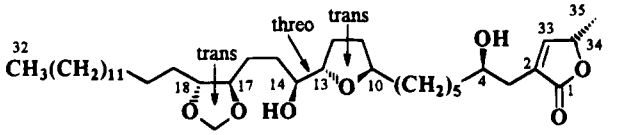
[a] Absolute configuration of carbinol center.



gigantetrocin A (**9**)



14,17-formaldehyde acetal derivative of gigantetrocin A (**20**)



17,18-formaldehyde acetal derivative of gigantetrocin A (**21**)

Figure 4.

than to form a seven-membered acetal ring. The increase of molecular weights to 608 in both products (HR-FABMS: obsd 609.4730 in **20** and 609.4714 in **21** for MH^+, calcd 609.4730) and the appearance of the acetal proton signals (at δ 5.28 and 4.62 in **20** and at δ 4.96 in **21**) in their respective ^{1}H NMR spectra confirmed the formation of the acetal moiety in both **20** and **21**. The structures of **20** and **21** were further confirmed by their ^{1}H and ^{13}C NMR and COSY spectra and the EIMS data of their TMSi derivatives. The fragment peak at *m/z* 299 in the EIMS of **20**, in particular, proved the formation of its acetal at C-14 and C-17, and that at *m/z* 309 in the EIMS of **21** placed its acetal at C-17 and C-18. The acetal protons appeared as a doublet at δ 5.28 and 4.62 in the ^{1}H NMR spectrum of **20** and indicated the *cis* configuration for the newly formed acetal ring, and the acetal protons presented a singlet at δ 4.96 in the ^{1}H NMR spectrum of **21** and indicated the *trans* configuration for the newly formed acetal ring; the latter data suggested that the vicinal diol at C-17,18 in **9** possesses a *threo* configuration; the same result was previously obtained by analysis of the ^{1}H NMR of the acetonide derivative of **9**.[1b] The (*S*)- and (*R*)-MTPA esters of **21** were then prepared, and C-14 was determined to have the *S* absolute configuration. Since the relative stereochemical relationship between C-14 and C-17 was already revealed to be either *S*/*R* or *R*/*S*, the absolute configuration at C-17 was concluded to be *R*. The absolute configuration at C-4 was also *R*, as indicated by the $\Delta\delta_H$ ($\delta_S - \delta_R$) values in Table 5. The structure of **9**, showing the proposed absolute configuration (4*R*,10*R*,13*S*,14*S*,17*R*,18*R*,36*S*), is illustrated in Figure 4.

Careful readers may have noticed that the $\Delta\delta_H$ ($\delta_S - \delta_R$) value of H-13 of the (*S*)- and (*R*)-MTPA esters of **21** (Table 5) is −0.02 ppm and not a positive value as it is supposed to be. Nevertheless, the conclusion of the *R* absolute configuration at C-14 seems to be unambiguous since all of the other $\Delta\delta_H$ ($\delta_S - \delta_R$) values of the protons on both sides of C-14 consistently showed either positive (on the THF ring side) or negative (on the chain and acetal ring side) values. The converse value of H-13 might be caused from different conformations of the THF and acetal rings in the (*S*)- and (*R*)-MTPA esters of **21**. The $\Delta\delta_H$ ($\delta_S - \delta_R$) values of such THF flanking protons (H-23 in the (*S*)- and (*R*)-MTPA esters of **11**–**13**, H-16 in the (*S*)- and (*R*)-MTPA esters of **19**, and H-17 in the (*S*)- and (*R*)-MTPA esters of **22**) were found to be about

Table 5. ^{1}H NMR Data of 20, 21, and (*S*)- and (*R*)-Per-MTPA-21 [δ ppm (J = Hz)]

proton	20	21	(*S*)-21	(*R*)-21	$\Delta\delta_H(\delta_S - \delta_R)$
35	1.44 d (7.0)	1.43 d (7.0)	1.29 d (7.0)	1.32 d (7.0)	neg
34	5.06 qq	5.07 qq	4.86 qq	4.91 qq	neg
33	7.19 q	7.19 q	6.72 q	6.98 q	neg
3a	2.53 ddt	2.54 ddt	2.60	2.68	neg
b	2.40 ddt	2.40 ddt	2.58	2.60	neg
4	3.84 m	3.86 m	5.31 m	5.37 m	*R*[a]
5	1.48 m	1.48 m	1.69 m	1.65 m	pos
6–8	1.40–1.20	1.40–1.20	1.40–1.20	1.40–1.20	
9	1.53	1.53	1.47	1.43 m	pos
10	3.96 m	3.88 m	3.88 m	3.78 m	pos
11a	2.02 m	2.02 m	2.01 m	1.88 m	pos
11b	1.48 m	1.52 m	1.47 m	1.40 m	pos
12a	1.96 m	1.98 m	2.02 m	1.96 m	pos
12b	1.64 m	1.61 m	1.60 m	1.56 m	pos
13	4.00 q	3.78 q	4.00 q	4.02 q	~0.02
14	3.61 m	3.40 m	5.08 m	5.09 m	*S*[a]
15a	1.88 m	1.86 m	1.79 m	1.90 m	neg
15b	1.80 m	1.40 m	1.50 m	1.68 m	neg
16	1.85 m	1.57 m	1.50 m	1.55 m	neg
17	3.52 m	3.52 m	3.30 m	3.46 m	neg
18	3.47 m	3.52 m	3.32 m	3.47 m	neg
36a	5.28 d (7.5)	4.96 s	4.92 s	4.96 s	neg
36b	4.62 d (7.5)		4.89 s	4.93 s	neg
19	1.41 m	1.57 m	1.43 m	1.47 m	neg
20–31	1.40–1.20	1.40–1.20	1.40–1.20	1.40–1.20	
32	0.880 t (7.0)	0.880 t (7.0)	0.880 t (7.0)	0.880 t (7.0)	
MeO-4			3.51 s	3.50 s	
MeO-14			3.66 s	3.53 s	
Ar-10H			7.60–7.37	7.60–7.37	

[a] Absolute configuration of carbinol center.

Table 6. ^{1}H NMR Data of 22 and (*S*)- and (*R*)-Per-MTPA-22 [δ ppm (J = Hz)]

proton	22	(*S*)-22	(*R*)-22	$\Delta\delta_H(\delta_S - \delta_R)$
35	1.43 d (7.0)	1.28 d (7.0)	1.31 d (7.0)	neg
34	5.06 qq	4.86 qq	4.91 qq	neg
33	7.19 q	6.72 q	6.97 q	neg
3a	2.53 ddt	2.60	2.68	neg
b	2.40 ddt	2.58	2.60	neg
4	3.84 m	5.31 m	5.37 m	*R*[a]
5	1.47 m	1.65 m	1.61 m	pos
6	1.40–1.20	1.30 m	1.24 m	pos
7–8	1.40–1.20	1.40–1.20	1.40–1.20	
9	1.58 m	1.72, 1.55 m	1.73 m	
10	3.66 m	3.60 m	3.62 m	
11	1.80 m	1.80, 1.66 m	1.87 m	
12	1.80 m	1.72, 1.54 m	1.73 m	
13	3.66 m	3.62 m	3.63 m	
14	3.99 q	3.92 q	4.02 q	neg
15a	1.98 m	1.75 m	1.92 m	neg
b	1.70 m	1.65 m	1.79 m	neg
16a	1.98 m	1.91 m	2.03 m	neg
b	1.64 m	1.52 m	1.60 m	neg
17	3.84 q	4.08 q	4.08 q	~0
18	3.39 q	5.05 q	5.05 q	*R*[a]
19	1.40 m	1.60 m	1.48 m	pos
20	1.40–1.20	1.30 m	1.14 m	pos
21–31	1.40–1.20	1.40–1.20	1.40–1.20	
32	0.880 t (7.0)	0.880 t (7.0)	0.882 t (7.0)	
36a	5.16 d (7.5)	5.11 d (7.5)	5.11 d (7.5)	
b	4.61 d (7.5)	4.54 d (7.5)	4.53 d (7.5)	
MeO-4		3.52 s	3.50 s	
MeO-18		3.56 s	3.54 s	
Ar-10H		7.60–7.37	7.60–7.37	

[a] Absolute configuration of carbinol center.

zero (Tables 1, 2, and 4–6) if the carbinol centers have a *threo* relative stereochemical relationship with the THF ring. Therefore, we strongly urge future investigators to determine as many as possible of the $\Delta\delta_H$ ($\delta_S - \delta_R$) values of the protons on both sides of the carbinol centers, especially those of the THF methylene protons, to achieve more confident assignments of the absolute configurations of the carbinol centers.

The relative stereochemistries of the vicinal diols in Annonaceous acetogenins are usually determined by observing the chemical shifts of the acetonyl methyl protons in the ^{1}H NMR spectra of their acetonide derivatives; *i.e.*, one singlet at δ 1.37 indicates *threo* and two separate singlets at δ 1.43 and 1.33 indicate *erythro*.[1b,23] The acetonide derivative decomposes very easily, and the decomposition can occur during the purification procedure or even in the NMR tube in $CDCl_3$ during spectral analyses. In some cases, the proton signals of the acetonyl methyls are not distinct since they are usually overlapped with the large envelope of methylene proton signals. However, the formaldehyde acetal derivatives are relatively stable, and the acetal proton signals, located downfield from the aliphatic methylene proton signals, can be easily observed. Thus, formaldehyde acetal derivatization can also be used as a convenient method for determination of the relative stereochemistry of vicinal diols.

Goniothalamicin (**10**, Figure 5) is a common mono-THF acetogenin, whose absolute stereochemistry has not been previously solved.[1,12] A particular problem in determination of the absolute configuration of goniothalamicin is focused on C-10. The formaldehyde acetal derivative (**22**) was prepared, and the analyses of the $\Delta\delta_H$ ($\delta_S - \delta_R$) values of the (*S*)- and (*R*)-MTPA esters of **22** resulted in the determination of the absolute configuration at C-18 to be 18*R*; those at C-13, C-14, and C-17 were then all solved to be *R* by tracing their relative stereochemistries. The absolute configuration at C-10 was subsequently concluded to be also 10*R*, since an *R*/*R* or an *S*/*S* relative configuration relationship, between C-10 and C-13, had been revealed by the observation of the acetal protons which were presented as a pair of doublets at δ 5.16 and 4.61 (J = 7.5 Hz) in the ^{1}H NMR spectrum of **22** (Table 6). The Mosher esters also demonstrated

(23) Gu, Z.-M.; Fang, X.-P.; Zeng, L.; Kozlowski, J. F.; McLaughlin, J. L. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 473.

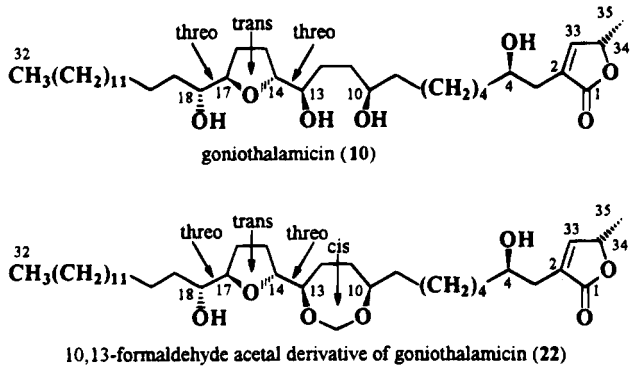


Figure 5.

that the absolute configuration at C-4 was *R*, as usual with all acetogenins that have a 4-OH. The structure of **10**, showing the proposed absolute configuration (4*R*,-10*R*,13*R*,14*R*,17*R*,18*R*,36*S*), is illustrated in Figure 5.

These formaldehyde acetal derivatives of the Annonaceous acetogenins, like their parent compounds, are highly bioactive in the brine shrimp lethality test (BST)[24] and potently cytotoxic against human solid tumor cell lines in culture[25-27] (Table 7). Most of the acetal derivatives showed, to some extent, higher cytotoxic potencies and selectivities than their parent acetogenins; this conclusion was further confirmed by a parallel of enhanced inhibition of oxygen uptake by rat liver mitochondria (RMB);[2] these results demonstrated that the site of biological action of the formaldehyde acetal derivatives is the same as that of their parent acetogenins and that some new mode of biological action was not created by the acetalations. These observations may be helpful in future formulation efforts to modify the biological delivery and persistence of the acetogenins as drug products.

Experimental Section

General Information. Bullatanocin (**1**),[1b,8] (2,4-*cis* and *trans*)-bullatanocinones (**2** and **3**),[1b,8] bullatalicin (**4**),[6,9] (2,4-*cis* and *trans*)-bullatalicinones (**5** and **6**),[6,17] squamocin (**8**),[1a,13] gigantetrocin A (**9**),[1b,11] and goniothalamicin (**10**)[1a,12] were available as isolated in our laboratory from several plant species in the Annonaceae. Squamostatin A (**7**)[6,10] was isolated from *Annona squamosa* and provided by Bayer AG, Germany.

All proton chemical shifts > δ 2.10 were determined directly from the 1D ^{1}H NMR spectra, whereas, because of the large degree of overlap among the resonances, all shifts < δ 2.10 were carefully estimated from the centers of the relevant 2D COSY off-diagonal peaks. All of the reagents are Aldrich products.

Preparation and Purification of Formaldehyde Derivatives. To Me_3SiCl (100 mg, in 3 mL of CH_2Cl_2) was added Me_2SO (100 mg in 2 mL of CH_2Cl_2), and the mixture was allowed to stand at room temperature for about 1 h until a white precipitate appeared. The CH_2Cl_2 was decanted, and the white precipitate was quickly washed with 1 mL of CH_2Cl_2. To this precipitate, the starting acetogenin (30–60 mg, in 5 mL of CH_2Cl_2) was added with stirring at room temperature for 36–72 h, until almost all of the starting material had disappeared as determined by TLC. The mixture was washed using 1% $NaHCO_3$ (5 mL) and H_2O (2 × 5 mL), and the CH_2Cl_2 layer was dried *in vacuo*. The products were purified by normal phase open column chromatography (0.5% MeOH in $CHCl_3$) or HPLC [5–10% MeOH:THF (9:1) in hexane]. Yields were 25–40%, and unreacted starting materials were often recovered.

Preparation and Purification of Mosher Esters. To an acetogenin or a formaldehyde derivative of an acetogenin (0.5–1 mg, in 0.3 mL of CH_2Cl_2) were sequentially added pyridine (0.2 mL), 4-(dimethylamino)pyridine (0.5 mg), and 25 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride.[18] The mixture was stirred at room temperature for 4 h and passed through a disposable pipet (0.6 × 6 cm) containing silica gel (60–200 mesh) and eluted with 3 mL of CH_2Cl_2. The CH_2Cl_2 residue, dried *in vacuo*, was redissolved in CH_2Cl_2 and washed using 1% $NaHCO_3$ (5 mL) and H_2O (2 × 5 mL); the CH_2Cl_2 layer was dried *in vacuo* to give the (*S*)-Mosher esters. Using (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride[19] gave the (*R*)-Mosher esters. Both yields were typically higher than 90%.

TMSi Derivatizations. Compounds **11–21** (*ca.* 0.3 mg of each) were treated with *N,O*-bis(trimethylsilyl)acetamide (20 μL) and pyridine (2 μL) and heated at 70 °C for 30 min to yield the respective tetra-TMSi derivatives.

Bioassays. The brine shrimp lethality test (BST), on newly hatched naupleii, was performed in our laboratory as previously described.[24] Seven day cytotoxicities against human solid tumor cells were measured at the Cell Culture Laboratory, Purdue Cancer Center, for the A-549 lung carcinoma,[25] MCF-7 breast carcinoma,[26] and HT-29 colon adenocarcinoma.[27] The respiratory functions of rat liver mitochondria (RMB) were measured polarographically by determination of their rates of oxygen consumption after the addition of acetogenins or their derivatives.[2c,d] The bioassay data are summarized in Table 7 for compounds **1-22**.

16,19–Formaldehyde Acetal Derivative of Bullatanocin (11). Sixty mg of bullatanocin (**1**) was converted into 18 mg of **11** (yield: 30%) as a white wax. HRFABMS: obsd. 651.4828, calcd 651.4836, for $C_{38}H_{67}O_8$ (MH^+). ^{1}H NMR: see Table 1. ^{13}C NMR δ: 95.8 (C-38, acetal carbon), 81.0 (C-19), 80.3 (C-16).

4,24-TMSi Derivative of 11. EIMS *m/z* (rel int): 624 (12.5), 594 (28.6), 563 (11.4), 551 (43.1), 533 (11.1), 521 (27.0), 503 (36.3), 485 (16.3), 493 (14.8), 473 (18.1), 461 (18.9), 455 (28.8), 420 (22.1), 413 (39.0), 407 (22.6), 395 (24.6), 391 (17.1), 381 (53.2), 352 (22.9), 339 (33.1), 335 (25.2), 323 (33.2), 313 (45.7), 309 (30.6), 299 (37.3), 293 (37.9), 291 (33.5), 275 (33.8), 273 (30.7), 269 (44.2), 257 (25.8), 249 (24.1), 243 (70.8), 239 (29.6), 229 (33.7), 227 (35.2), 223 (47.7), 213 (69.4), 199 (32.4), 191 (36.6), 184 (69.1), 169 (100.0).

4,24-(*S*)- and -(*R*)-MTPA Esters of 11. White colorless oils. ^{1}H NMR: see Table 1.

16,19-Formaldehyde Acetal Derivatives of (2,4-*cis* and *trans*)-Bullatanocinones (12 and 13). Fifty mg of (2,4-*cis* and *trans*)-bullatanocinones (**2** and **3**) was converted into 15 mg of **12** and **13** (yield: 30.0%) as a white oil. HRFABMS: obsd 651.4836, calcd 651.4836, for $C_{38}H_{67}O_8$ (MH^+). ^{1}H NMR: see Table 2.

24-TMSi Derivatives of 12 and 13. EIMS *m/z* (rel int): 745 (100.0, M + Na), 479 (6.6), 449 (3.3), 413 (6.5), 313 (9.8), 309 (27.9), 291 (3.2) 243 (100.0), 141 (27.0).

24-(*S*)- and -(*R*)-MTPA Esters of 12 and 13. White colorless oils. ^{1}H NMR: see Table 2.

16,19-Formaldehyde Acetal Derivative of Bullatalicin (14). Eighty mg of bullatalicin (**4**) was converted into 22 mg of **14** (yield: 27.5%) as a white wax. HRFABMS: obsd 651.4817, calcd 651.4836, for $C_{38}H_{67}O_8$ (MH^+). ^{1}H NMR: see Table 1. ^{13}C NMR δ: 95.7 (C-38, acetal carbon), 81.7 (C-19), 80.3 (C-16).

4,24-TMSi Derivative of 14. EIMS *m/z* (rel int): 624 (26.6), 594 (38.3), 563 (13.8), 551 (100.0), 533 (16.0), 521 (35.2), 503 (67.2), 485 (7.0), 455 (7.7), 420 (8.0), 413 (21.9), 407 (9.7), 395 (12.0), 391 (7.4), 381 (64.7), 352 (11.2), 339 (12.6), 313

(24) (a) Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobson, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 32. (b) McLaughlin, J. L. In *Methods in Plant Biochemistry*; Hostettmann, K., Ed.; Academic Press: London, 1991; Vol. 6, pp 1–33.

(25) Giard, D. J.; Aronson, S. A.; Todaro, G. J.; Arnstein, P.; Kersey, J. H.; Dosik, H.; Parks, W. P. *J. Natl. Cancer Inst.* **1973**, *51*, 1417.

(26) Soule, H. D.; Vazquez, J.; Long, A.; Albert, S.; Brennan, M. *J. Natl. Cancer Inst.* **1973**, *51*, 1409.

(27) Fogh, J.; Trempe, G. New Human Tumor Cell Lines. In *Human Tumor Cells, in vitro*; Fogh, J., Ed.; Plenum Press: New York, 1975; p 115.

Table 7. Bioactivities of Compounds 1–22[a]

compd	BST[b] LC_{50} (μg/mL)	A-549[c] ED_{50} (μg/mL)	MCF-7[d] ED_{50} (μg/mL)	HT-29[e] ED_{50} (μg/mL)	RMB[f] IC_{50} (*n* mol/Lt/mg protein)
1	4.30×10^{-1}	5.15×10^{-10}	2.42×10^{-2}	1.66×10^{-11}	50.7
11	6.50×10^{-3}	1.43×10^{-11}	2.22×10^{-1}	2.07×10^{-13}	11.3
2 and **3**	2.80×10^{-1}	6.43×10^{-4}	2.93×10^{-1}	2.34×10^{-8}	61.1
12 and **13**	1.50×10^{-2}	6.77×10^{-5}	1.28	7.01×10^{-13}	28.3
4	1.20	1.22×10^{-10}	2.82×10^{-1}	6.48×10^{-8}	19.2
14	4.90×10^{-2}	1.28×10^{-13}	1.85×10^{-2}	9.10×10^{-13}	27.8
5 and **6**	4.70×10^{-1}	5.62×10^{-5}	1.57×10^{-3}	2.42×10^{-13}	23.0
15 and **16**	3.80×10^{-2}	3.69×10^{-9}	6.39×10^{-2}	2.25×10^{-13}	20.5
7	2.80	2.68×10^{-4}	7.20×10^{-5}	2.04×10^{-11}	
17	10.2	1.78	1.00×10^{-3}	2.89×10^{-7}	
18	7.00×10^{-3}	4.87×10^{-9}	1.09	3.03×10^{-13}	
8	2.00×10^{-2}	2.49×10^{-12}	3.03×10^{-2}	1.91×10^{-13}	21.5
19	4.40×10^{-3}	2.37×10^{-10}	6.95×10^{-1}	2.89×10^{-13}	421.1
9	6.00×10^{-1}	4.52×10^{-8}	3.55×10^{-4}	3.06×10^{-12}	287.1
20		7.98×10^{-4}	4.61×10^{-1}	7.30×10^{-13}	
21	7.50×10^{-3}	6.95×10^{-2}	2.58×10^{-1}	3.57×10^{-12}	290.3
10	37.0	7.11×10^{-6}	5.73×10^{-6}	1.71×10^{-9}	554.8
22	5.38×10^{-2}	1.05	4.09×10^{-4}	2.19×10^{-5}	203.1
adriamycin[g]	8.00×10^{-2}	4.19×10^{-3}	4.01×10^{-1}	3.55×10^{-2}	
rotenone[h]	1.23×10^{-2}	1.66×10^{-3}	4.08×10^{-5}	2.55×10^{-8}	34.8

[a] All of the samples were tested in the same run in each bioassay except in the RMB, in which the parent compounds and their respective acetal derivatives were tested in the same day. [b] Brine shrimp lethality test.[24] [c] Human lung carcinoma.[25] [d] Human breast carcinoma.[26] [e] Human colon adenocarcinoma.[27] [f] Rat mitochondrial bioassay.[2] [g] Positive antitumor control standard. [h] Positive pesticide control standard.

(30.2), 309 (9.5), 293 (12.7), 291 (15.0), 275 (30.7), 273 (12.0), 269 (26.5), 243 (100.0), 229 (13.1), 227 (11.1), 223 (16.6), 213 (57.2).

4,24-(*S*)- and -(*R*)-MTPA Esters of 14. White colorless oils. ^{1}H NMR: see Table 1.

16,19-Formaldehyde Acetal Derivatives of (2,4-*cis* and *trans*)-Bullatalicinones (15 and 16). Forty mg of (2,4-*cis* and *trans*)-bullatalicinones (**5** and **6**) was converted into 13 mg of **12** and **13** (yield: 32.5%) as a white oil. HRFABMS: obsd 651.4823, calcd 651.4836, for $C_{38}H_{67}O_8$ (MH^+). ^{1}H NMR: see Table 2.

24-TMSi Derivatives of 15 and 16. EIMS *m/z* (rel int): 722 (16.4, M^+), 479 (4.1), 449 (3.6), 413 (4.4), 383 (14.0), 367 (33.5), 339 (20.7), 313 (13.7), 309 (32.4), 291 (6.4) 269 (16.6), 243 (75.7), 213 (11.7), 141 (18.4).

24-(*S*)- and -(*R*)-MTPA Esters of 15 and 16. White colorless oils. ^{1}H NMR: see Table 2.

Formaldehyde Acetal Derivatives of Squamostatin A. Nine mg of squamostatin A (**7**) was converted into 1 mg of **16,19- and 24,28-bis-formaldehyde acetal derivative (17) of 7** [yield 11.1%; white colorless oil; HRFABMS obsd 663.4852, calcd 663.4836, for $C_{39}H_{67}O_8$ (MH^+)] and 2.5 mg of **16,19-mono-formaldehyde acetal derivative (18) of 7** [yield 27.8%; white wax; HRFABMS obsd 651.4828, calcd 651.4836, for $C_{38}H_{67}O_8$ (MH^+)]. ^{1}H NMR of **17** and **18**: see Table 3.

24,28-(*S*)- and -(*R*)-MTPA Esters of 18. White colorless oils. ^{1}H NMR: see Table 3.

24,28-Formaldehyde Acetal Derivative of Squamocin (19). Sixty-five mg of squamocin (**8**) was converted into 17 mg of **19** (yield: 28.3%) as a white oil. HRFABMS: obsd 635.4842, calcd 635.4887, for $C_{38}H_{67}O_7$ (MH^+). ^{1}H NMR: see Table 4.

15-TMSi Derivative of 19. EIMS *m/z* (rel int): 573 (5.6), 543 (10.1), 507 (15.2), 455 (79.4), 437 (13.7), 435 (10.9), 367 (100.0), 365 (21.5), 361 (23.1), 339 (6.5), 309 (13.9), 293 (13.2), 269 (4.6), 252 (19.0), 199 (7.2), 185 (12.4), 169 (27.0).

15-(*S*)- and -(*R*)-MTPA Esters of 19. White colorless oils. ^{1}H NMR: see Table 4.

Formaldehyde Acetal Derivatives of Gigantetrocin A. Sixty mg of gigantetrocin A (**9**) was converted into 2.4 mg of **14,17-formaldehyde acetal derivative (20) of 7** [yield 4.0%; white wax; HRFABMS obsd 609.4730, calcd 609.4730, for $C_{36}H_{65}O_7$ (MH^+)] and 21 mg of **17,18-formaldehyde acetal derivative (21) of 9** [yield 35.0%; white wax; HRFABMS obsd 609.4714, calcd 609.4730, for $C_{36}H_{65}O_7$ (MH^+)]. ^{1}H NMR of **20** and **21**; see Table 5. ^{13}C NMR of **21** δ: 93.8 (C-36, acetal carbon), 81.6 (C-18), 81.5 (C-17).

4,18-TMSi Derivative of 20. EIMS *m/z* (rel int): 737 (8.8), 707 (8.0), 641 (3.8), 617 (4.8), 551 (3.0), 496 (21.2), 461 (22.6), 453 (12.6), 426 (43.2), 423 (7.7), 369 (18.0), 363 (8.2), 353 (100.0), 309 (24.6), 299 (84.2), 263 (13.8), 245 (19.3), 213 (48.2).

4,14-TMSi Derivative of 21. EIMS *m/z* (rel int): 753 (5.9, MH^+), 737 (6.7), 707 (2.0), 663 (5.3), 617 (3.2), 426 (84.6), 399 (7.2), 383 (28.7), 369 (33.0), 353 (100.0), 336 (10.7), 309 (64.5), 295 (7.4), 281 (18.1), 279 (18.8), 263 (19.7), 245 (26.0), 243 (18.8), 213 (32.4), 184 (58.4).

4,14-(*S*)- and -(*R*)-MTPA Esters of 21. White colorless oils. ^{1}H NMR: see Table 5.

10,13-Formaldehyde Acetal Derivative of Goniothalamicin (22). Fifty mg of goniothalamicin (**10**) was converted into 16 mg of **22** (yield: 32.0%) as a white wax. HRFABMS: obsd 609.4730, calcd 609.4730, for $C_{36}H_{65}O_7$ (MH^+). ^{1}H NMR: see Table 6. ^{13}C NMR δ: 95.2 (C-36, acetal carbon), 81.1 (C-13), 80.0 (C-10).

4,18-TMSi Derivative of 22. EIMS *m/z* (rel int): 737 (1.2), 707 (2.7), 641 (5.9), 541 (4.2), 524 (12.5), 496 (8.7), 453 (13.5), 425 (41.0), 423 (16.0), 385 (15.4), 383 (8.8), 369 (22.8), 353 (24.0), 299 (87.7), 213 (33.5).

4,18-(*S*)- and -(*R*)-MTPA Esters of 22. White colorless oils. ^{1}H NMR: see Table 6.

Acknowledgment. This investigation was supported by R01 Grant No. CA30909 from the National Cancer Institute, National Institutes of Health. Thanks are due to the Purdue Cell Culture Laboratory, Purdue Cancer Center.

Supplementary Material Available: Copies of ^{1}H spectra of **11**–**22** and their (*S*)- and (*R*)-Mosher esters (13 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.