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ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · FEBRUARY 2008

Impact Factor: 3.8 · DOI: 10.1021/np0704351 · Source: PubMed

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Semisynthetic and Biotransformation Studies of (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-Cembratriene-4,6-diol

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Received August 16, 2007

Tobacco-derived (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (**1**) and (1*S*,2*E*,4*R*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (**2**) were first shown to display potential antitumor-promoting activity in the mid-1980s. However, very little is currently understood regarding the structural activity relationships of tobacco cembranoids. The aim of this present study was to explore antiproliferative activity of various derivatives of (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (**1**) using semisynthetic and biotransformation approaches. Derivatives of **1** include esterified, oxidized, halogenated, and nitrogen- and sulfur-containing compounds (**3**–**17**). Biotransformation of **1** using *Mucor ramannianus* ATCC 9628 and *Cunninghamella elegans* ATCC 7929 afforded the known 10*S*,11*S*-epoxy analogue of **1** (**4**) as the main metabolite. Biotransformation of the 6-*O*-acetyl analogue (**3**) using the marine symbiotic *Bacillus megaterium* strain MO31 afforded (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*,10*R*)-2,7,11-cembratriene-4,6,10-triol (**18**). (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*,13*R*)-2,7,11-Cembratriene-4,6,13-triol-6-*O*-acetate (**6**), (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*,13*S*)-2,7,11-cembratriene-4,6,13-triol-6-*O*-acetate (**7**), the rearranged α-ketol (1*S*,2*E*,4*S*,7*Z*,11*E*)-2,7,11-cembratrien-4-ol-6-one (**11**), and the secocembranoid **12** showed antiproliferative activity against highly malignant +SA mammary epithelial cells with an IC_{50} range of 15–30 μM.

The leaf and flower cuticular wax of most *Nicotiana* species contains high amounts of cembranoid diterpenes.[1] They possess 14-membered macrocyclic rings substituted by an isopropyl residue at C-1 and by three symmetrically disposed methyl groups at positions C-4, C-8, and C-12. The two most abundant epimeric tobacco cembranoids, (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (**1**) and (1*S*,2*E*,4*R*,6*R*,-7*E*,11*E*)-2,7,11-cembratriene-4,6-diol (**2**), were among the first reported.[1,2] The structure and absolute stereochemistry of **2** was based on X-ray crystallography, while the structure of **1** was based on chemical degradation and correlation with 2,7,12(20)-cembratriene-4,6,11-triol, which was confirmed by X-ray crystallography.[1–4] Both compounds are key flavor ingredients in tobacco.[1,2] Biodegradation of **1** and **2** during cure fermentation or flue curing of tobacco leaf produces a range of flavor compounds having from 8 to 19 carbon skeletons.[1,2,5]

Tobacco cembranoids block the expression of the behavioral sensitization to nicotine and inhibit neuronal acetylcholine receptors in rats, suggesting a possible use of these compounds for the treatment of nicotine addiction.[6] Tobacco cembranoids also show insecticidal, prostaglandin, plant growth, and fungal spore germination inhibitory activities.[1,2,5,7]

Cembranoids **1** and **2** from cigarette smoke condensate were reported as antitumor-promoter ingredients in 1985.[8] Later, **1** and **2** were reported to inhibit the induction of Epstein–Barr virus (EBV) early antigen by the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in lymphoblastoid Raji cells.[9] Their activity level was similar to that displayed by retinoic acid, an active form of vitamin A.[9] Cembranoids **1** and **2** were also found to inhibit the induction of ornithine decarboxylase (OD) activity by TPA in the mouse epidermis.[10] Cembranoids **1** and **2** also inhibited the promotional effects of TPA on skin tumor formation initiated by 7,12-dimethylbenz[*a*]anthracene (DMBA) in mice.[10] In these initiation−promotion experiments, **1** was much more active than its epimer **2**. Application of 3.3 μM **1** for 40 min prior to TPA treatment resulted in a 53% reduction in the incidence of papillomas in mice.[10] A dose of 50 μM **1** was able to inhibit 28.4% of $^{32}P_i$ incorporation into phospholipids of TPA-stimulated HeLa cells without any interaction with calmodulin. Unlike many anticancer agents, **1** was not found to be cytotoxic even at very high doses (100 μM).[10] However, **1** did not inhibit the specific binding of ^{3}H-TPA to mouse epidermal particulate fraction even at doses up to 1 mM *in vitro* and 10 mg *in vivo*.[10] Cembranoid **1** was found to induce a dose-responsive inhibition of TPA-stimulated phosphorylation of the 47K-Da human platelet protein and displayed an IC_{50} value of 100 μM.[10] These results suggested that **1** does not directly compete with or modulate TPA binding on the protein kinase C (PKC) receptor.

Several semisynthetic reactions were reported for tobacco cembranoids.[11] These included oxidations of $\Delta^{7,8}$ and $\Delta^{11,12}$, allylic oxidations around $\Delta^{11,12}$, oxidation of a C-6 secondary alcohol to a keto group, and acid-catalyzed rearrangements.[11] These reactions were conducted to aid the structure elucidation or correlate different compounds.[11] Only a single structure–activity relationship (SAR) study was carried out to optimize tobacco cembranoids' antitumor-promoting activity.[10] This study highlighted the need for better understanding of SAR through the use of additional semisynthetic modifications. Therefore, the present study subjected the readily available tobacco cembranoid **1** to semisynthetic and biotransformation methods in an attempt to further optimize anticancer activity. Acylation of C-6 of **1** and **2** using acetic, propionic, butyric, valeric, and benzoic acid anhydrides reduced the EBV early antigen formation inhibitory activity and increased the cytotoxicity, suggesting the importance of a free C-6 hydroxy for the activity.[10] Cytotoxicity increased with the increase of acyl carbon number.[10] Oxidation of the C-6 alcohol of **1** and **2** to β-hydroxyketones slightly reduced the activity by increasing the IC_{50}, which further confirmed the importance of a hydrogen bonding donor pharmacophore at C-6. This was also evident when the dehydration and rearranged products of **1**, were found to be almost inactive.[10] In this study, semisynthetic carbamoylation of C-6 in **1** was conducted to add more hydrogen bonding donor and acceptor pharmacophores at this key position, which may enhance the binding affinity of the products toward its target protein(s). Similarly, biotransformation and semisynthetic hydroxylation studies were carried out to test the effect of possible additional allylic hydroxylation. Halogen substitution in the mac-

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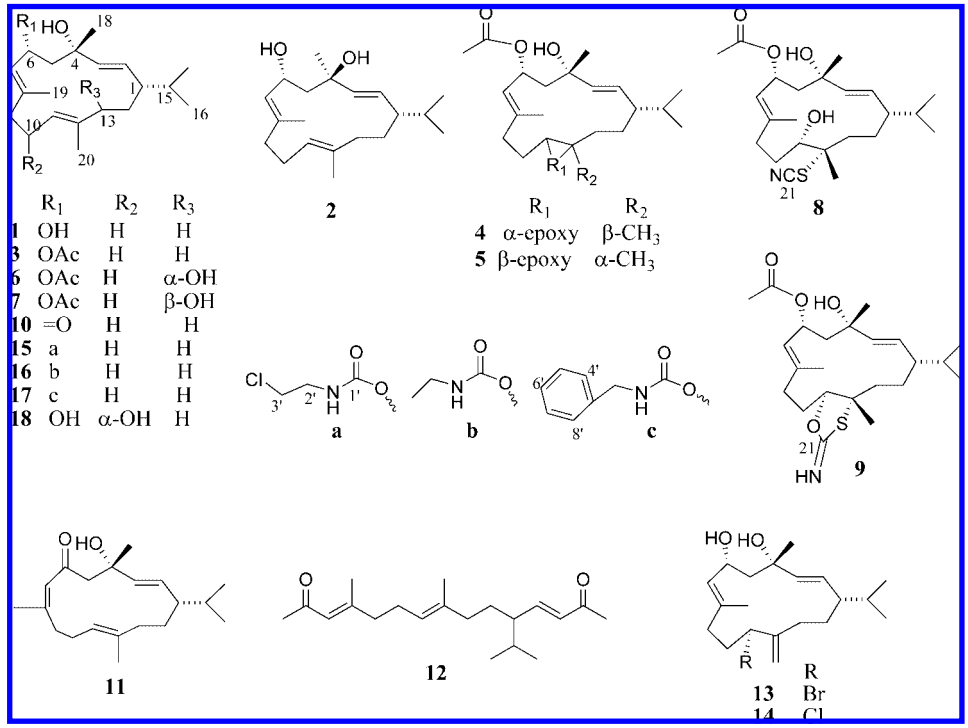
10.1021/np0704351 CCC: $40.75
Published on Web 01/05/2008



rocyle aimed at the examination of the effect of electron-withdrawing groups (+σ) like Cl or Br on the anticancer activity.

Catalytic reduction of **1** resulted in a totally inactive hexahydro analogue.[10] Hence, macrocyclic double bonds proved essential for tobacco cembranoid EBV early antigen formation induction inhibitory activity.[10] The 11*S*,12*S*-epoxy analogue of **1** was devoid of activity, indicating the importance of $\Delta^{11,12}$ for activity.[10] C-11 or C-12 hydroperoxide analogues of **1** were also found to be much less active and more cytotoxic than the parent compound, further confirming the significance of $\Delta^{11,12}$ for the activity.[10] Reaction of $NH_4SCN/SbCl_3$ with the C-7/C-8 oxirane group in the related marine cembranoid sarcophine afforded the β-hydroxy thiocyanate and 1,3-oxathiolan-2-imine with enhanced antiproliferative activity.[12] The same reaction was conducted using **4** as a starting material to test whether the activity would also be increased and to test whether the C═NH group in the 1,3-oxathiolan-2-imine will be able to replace the $\Delta^{11,12}$ pharmacophore. In addition, studies were conducted to determine if antiproliferative activity could be increased through enhancing the binding affinity toward the target protein of **1**, since sulfur and oxygen may act as hydrogen bonding acceptors while the NH (in 1,3-oxathiolan-2-imine) and C-11 hydroxy group (in β-hydroxy thiocyanate) may act as hydrogen bonding donors. Condensation reaction of NH_4SCN with α-ketol (1*S*,2*E*,4*S*,7*E*,11*E*)-2,7,11-cembratrien-4-ol-6-one (**10**) was also conducted to prepare the α,β-unsaturated imine hydrothiocyanate to replace the C-6 hydroxy with this polar group and determine the effect of this modification on anticancer activity as compared to the parent compound.

Results and Discussion

High amounts of tobacco cembranoids **1** and **2** were isolated from fresh *Nicotiana tabacum* leaf powder. Their identification was based on extensive analyses of NMR data and comparison with the literature.[1–3,11]

Allylic hydroxylation of **1** using SeO_2 was unsuccessful. Therefore, the more stable 6-*O*-acetate (**3**) was prepared from **1** using acetic anhydride/pyridine and was subsequently used for oxidations with SeO_2 under different conditions. Reaction of **3** with SeO_2 in 1,4-dioxane at room temperature in the presence of 30% H_2O_2 produced the 11*S*,12*S*- and 11*R*,12*R*-epoxide analogues of **3** (**4** and **5**, respectively) in a 2:1 ratio.[13] Reaction of **3** with SeO_2 in 1,4-dioxane, without H_2O_2, resulted in the known (1*S*,2*E*,4*S*,-6*R*,7*E*,11*E*,13*R*)-2,7,11-cembratriene-4,6,13-triol-6-*O*-acetate (**6**) and (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*,13*S*)-2,7,11-cembratriene-4,6,13-triol-6-*O*-acetate (**7**).[14] Identification of these compounds was based on extensive analysis of their NMR data and comparison with literature.[11,13,14]

The epoxide (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-2,7-cembradiene-11,12-epoxy-4,6-diol-6-*O*-acetate (**4**) was reacted with $NH_4SCN/SbCl_3$ to afford β-hydroxy thiocyanate (**8**) and 1,3-oxathiolan-2-imine (**9**) in 23.1% combined yields. The HRESMS of **8** showed a molecular ion peak at *m*/*z* 466.2341 $[M + Na]^+$, suggesting the molecular formula $C_{23}H_{37}NO_4S$. The IR spectrum of **8** showed a characteristic band for a SCN group at 2152 cm^{-1}. The ^1H and ^{13}C data of **8** (Tables 1 and 2) further suggested the replacement of the C-11/C-12 oxirane with a β-hydroxy thiocyanate functionality. The quaternary carbon at δ 111.9 was assigned the thiocyanate carbon C-21. The quaternary carbon at δ 62.8 was assigned C-12. This was based on its ^2J-HMBC correlation with the methyl singlet H_3-20 (δ 1.51). Similarly, the oxymethine proton doublet at δ 3.43 was assigned H-11. This was also based on the ^3J-HMBC correlation between the methyl singlet H_3-20 and the oxygenated methine carbon C-11 (δ 70.3). Proton H-11 shows a COSY correlation with H_2-10 (δ 1.58). The location of the thiocyanate group on C-12 rather than C-11 was also based on the carbon chemical shift of the quaternary C-12. The relative stereochemistry of C-11 and C-12 was established by extensive study of NOESY spectral data. The β-oriented H_3-18 (δ 1.31) showed a strong NOESY correlation with H-11, which in turn showed a NOESY correlation with H_3-20, suggesting a similar orientation.

The HRESMS data of **9** suggested a molecular formula identical to **8**. The ^1H and ^{13}C NMR spectral data of **9** (Tables 1 and 2) suggested the formation of a 1,3-oxathiolane ring at C-11/C-12. The methyl singlet H_3-20 (δ 1.37) showed a ^2J-HMBC correlation with C-12 (δ 64.0) and a ^3J-HMBC correlation with C-11 (δ 91.4). The exchangeable NH singlet at δ 7.20 showed a ^2J-HMBC correlation with the imine quaternary carbon C-21 (δ 187.5), confirming the 1,3-oxathiolane skeleton. The relative stereochemistry of C-11 and C-12 was deduced by a NOESY experiment. The β-oriented methyl singlets H_3-18 (δ 1.31) and H_3-20 (δ 1.37)

Table 1. ^{1}H NMR Data of **8**, **9**, **11**, **13**, and **14**[a]

	8	**9**	**11**	**13**	**14**
position	δ_H	δ_H	δ_H	δ_H	δ_H
1	1.88, m	1.76, m	1.61, m	1.70, m	1.70, m
2	5.75, dd (9.5, 15.4)	5.52, dd (9.1, 15.0)	5.57, dd (8.4, 15.4)	5.52, dd (9.1, 15.4)	5.52, dd (9.1, 15.4)
3	5.64, d (15.4)	5.35, d (15.0)	5.44, d (15.4)	5.41, d (15.4)	5.42, d (15.4)
5	2.07, dd (3.6, 16.8)	2.12, dd (5.4, 14.6)	2.72, dd (5.4, 14.6)	2.20, dd (5.8, 14.3)	2.22, dd (5.8, 14.3)
	1.97, dd (5.4, 16.8)	1.94, dd (2.9, 14.6)	2.42, dd (2.9, 14.6)	1.80, dd (2.5, 14.3)	1.80, dd (2.5, 14.3)
6	5.56, m	5.66, m		4.60, m	4.60, m
7	5.55, d (15.0)	5.35, d (15.0)	6.03, s	5.57, br d (9.5)	5.56, br d (9.8)
9	2.30, 2H, m	2.25, m; 1.82, m;	3.65, m; 2.02, m	2.00, 2H, m	1.85, 2H, m
10	1.58, 2H, m	1.45, 2H, m	2.22, m; 2.02, m	2.33, m 1.95, m	2.24, m 1.82, m
11	3.43, d (10.2)	4.44, dd (3.6, 11)	4.96, dd (6.2, 6.2)	4.46, dd (6.2, 10.2)	4.31, dd (6.2, 9.8)
13	1.71, m 1.39, m	1.35, 2H, m	2.10, m 1.90, m	2.29, m 1.90, m	2.15, m 1.75, m
14	1.85, m 1.35, m	1.73, 2H, m	1.49, 2H, m	1.65, m 1.45, m	1.65, m 1.35, m
15	1.40, m	1.65, m	1.51, m	1.59, m	1.60, m
16	0.79, 3H, d (6.6)	0.85, 3H, d (6.6)	0.79, 3H, d (6.6)	0.83, 3H, d (6.6)	0.85, 3H, d (6.6)
17	0.90, 3H, d (6.6)	0.88, 3H, d (6.6)	0.82, 3H, d (6.6)	0.86, 3H, d (6.6)	0.88, 3H, d (6.6)
18	1.31, 3H, s	1.31, 3H, s	1.27, 3H, s	1.28, 3H, s	1.26, 3H, s
19	1.65, 3H, s	1.72, 3H, s	1.84, 3H, d (1.4)	1.63, 3H, s	1.62, 3H, s
20	1.51, 3H, s	1.37, 3H, s	1.47, 3H, s	4.99, s; 5.18, s	5.00, s; 5.15, s
Ac-6	2.02, 3H, s	2.00, 3H, s			
NH		7.20, s			

[a] In $CDCl_3$, 400 MHz. Coupling constants (J) are in Hz.

Table 2. ^{13}C NMR Data of Compounds **8**, **9**, **11**, **13**, and **14**[a]

	8	**9**	**11**	**13**	**14**
position	δ_C	δ_C	δ_C	δ_C	δ_C
1	48.6, CH	50.4, CH	46.9, CH	48.6, CH	48.8, CH
2	132.5, CH	128.4, CH	131.2, CH	128.6, CH	128.4, CH
3	134.9, CH	140.1, CH	136.1, CH	140.3, CH	140.0, CH
4	72.6, qC	72.5, qC	72.6, qC	74.1, qC	74.2, qC
5	47.2, CH_2	46.6, CH_2	54.1, CH_2	46.8, CH_2	46.8, CH_2
6	68.3, CH	70.1, CH	202.2, qC	68.3, CH	68.4, CH
7	123.5, CH	123.5, CH	126.5, CH	128.3, CH	128.3, CH
8	141.1, qC	139.7, qC	160.3, qC	136.7, qC	136.9, qC
9	35.4, CH_2	34.6, CH_2	31.5, CH_2	37.0, CH_2	36.1, CH_2
10	23.2, CH_2	25.9, CH_2	25.7, CH_2	29.4, CH_2	29.4, CH_2
11	70.3, CH	91.4, CH	123.5, CH	56.8, CH	64.1, CH
12	62.8, qC	64.0, qC	134.7, qC	148.5, qC	148.0, qC
13	30.3, CH_2	33.8, CH_2	36.2, CH_2	35.6, CH_2	34.7, CH_2
14	27.5, CH_2	27.3, CH_2	29.0, CH_2	28.8, CH_2	28.6, CH_2
15	32.6, CH	32.8, CH	32.2, CH	33.3, CH	33.3, CH
16	20.7, CH_3	19.4, CH_3	20.0, CH_3	19.3, CH_3	19.3, CH_3
17	21.1, CH_3	21.0, CH_3	20.2, CH_3	21.0, CH_3	20.9, CH_3
18	29.7, CH_3	32.0, CH_3	30.0, CH_3	32.5, CH_3	32.4, CH_3
19	15.9, CH_3	15.9, CH_3	25.0, CH_3	15.6, CH_3	15.6, CH_3
20	27.2, CH_3	25.4, CH_3	15.0, CH_3	113.3, CH_3	113.5, CH_3
21	111.9, qC	187.5, qC			
Ac-6	170.3, qC	169.3, qC			
	21.4, CH_3	21.3, CH_3			

[a] In $CDCl_3$, 100 MHz. Carbon multiplicities were determined by DEPT135° or APT experiments. qC = quaternary, CH = methine, CH_2 = methylene, CH_3 = methyl carbons.

showed strong NOESY correlations with H-11 (δ 4.44), suggesting a similar stereo-orientation.

Reactions of epoxides with NH_4SCN usually afford the β-hydroxy thiocyanate intermediates followed by the end products thiiranes.[15] Although this reaction mechanism was proposed in the 1950s, the thiocyanohydrin intermediate was not isolated until recently.[15–17] Cyclic epoxides yield *trans* β-hydroxy thiocyanates under reasonably mild conditions and with proper catalysts.[16–18] These reactions are known to proceed with high regioselectivity, almost yielding anti-Markovnikov products.[16–18] However in sarcophine and (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-2,7-cembradiene-11,12-epoxy-4,6-diol-6-*O*-acetate (**4**), where the epoxide is attached to a 14-membered macrocycle, sulfur attack was exclusively on the more substituted carbon as per Markovnikov's rule, suggesting a carbocation intermediate followed by a thermodynamically stable product.[12,15] The *syn* addition of the nucleophile resulted in the *cis* β-hydroxy thiocyanate **8**.[12] The *cis* thiocyanohydrin intermediate is predicted to undergo a *cis* ring closure to form the stable 1,3-oxathiolan-2-imine (**9**), which represents a strained ring system.[12,15] The thiocyanohydrin intermediates of oxirane conversion with thiocyanate have been particularly difficult to isolate until the regio- and stereoselective synthesis of *trans* intermediates was reported.[15–18] It is worth noting that this reaction resulted in *cis* thiocyanohydrin and 1,3-oxathiolan-2-imine intermediates similar to sarcophine.[12] This selectivity could be attributed to the similarity of the cembranoid macrocycle ring size and conformation.[12]

Overnight reflux of **1** with CrO_3 in pyridine afforded the α-ketol (1*S*,2*E*,4*S*,7*E*,11*E*)-2,7,11-cembratrien-4-ol-6-one (**10**).[3,5] In an attempt to prepare the α,β-unsaturated imine hydrothiocyanate, α-ketol **10** was reacted with NH_4SCN in toluene.[19] Although the reaction did not proceed as planned, two major products, **11** and the known secocembranoid **12**, were isolated.[3,5,11]

The HRESMS of **11** showed a molecular ion peak at *m*/*z* 327.2288 $[M + Na]^+$, suggesting the molecular formula $C_{20}H_{32}O_2$. The ^{1}H and ^{13}C NMR data of **11** (Tables 1 and 2) were similar to the parent α-ketol **10** except for the segment C-7−C-9−C-19.[20,21] The methyl carbon C-19 (δ 25.0) of **11** was downfield shifted, compared with that of **10** (δ 15.7).[20,21] The methylene carbon at δ 31.5 was assigned C-9. This was based on the ^3J-HMBC correlation with the H_3-19 methyl doublet (δ 1.84). Carbon C-9 showed a HETCOR correlation with proton multiplets at δ 3.65 and 2.02 (H-9a and H9b). The downfield shifting of proton H-9a may be due to its location in the deshielding cone of $\Delta^{7,8}$. The double bond geometry of $\Delta^{7,8}$ was assigned as *Z* on the basis of the ^{13}C NMR chemical shift of the C-19 methyl and the strong NOESY correlation between H-7 (δ 6.03) and H_3-19.[3,5,11,21] Therefore, compound **11** was identified as (1*S*,2*E*,4*S*,7*Z*,11*E*)-2,7,11-cembratrien-4-ol-6-one.

Halogenations of **1** using *N*-bromosuccinimide (NBS) or *N*-chlorosuccinimide (NCS) in 10% aqueous acetone at room temperature afforded the halogenated cembranoids **13** and **14**, respectively.

Compound **13** showed the molecular ion peak at *m*/*z* 407.1564 $[M + Na]^+$, suggesting the molecular formula $C_{20}H_{33}BrO_2$. The mass spectrum of **13** also showed typical M and M + 2, 1:1, isotopic clusters characteristic for monobrominated compounds. The ^{1}H and ^{13}C NMR data of **13** (Tables 1 and 2) showed a different C-11−C-12−C-20 segment compared to those of the starting material **1**. The ^{1}H NMR spectrum of **13** showed signals for brominated and exocyclic methylene protons at δ 4.46 (H-11), 5.18 (H-20a), and 4.99 (H-20b), replacing the olefinic H-11 and the methyl H_3-20 in **1**, respectively. Proton H-11 showed a COSY

Table 3. ^{13}C and ^{1}H NMR Data of Compounds **15**–**17**[a]

	15		**16**		**17**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	46.4, CH	1.60, m	46.4, CH	1.60, m	46.4, CH	1.60, m
2	127.9, CH	5.29, dd (15.8, 9.2)	127.9, CH	5.30, dd (15.4, 9.2)	127.9, CH	5.30, dd (15.4, 8.8)
3	137.2, CH	5.31, d (15.8)	137.4, CH	5.32, d (15.4)	137.3, CH	5.31, d (15.4)
4	72.4, qC		72.4, qC		72.4, qC	
5	50.8, CH_2	1.95, 2H, m	50.9, CH_2	1.95, 2H, m	50.9, CH_2	1.99, 2H, m
6	69.7, CH	5.40, dd (10.5, 9.9)	69.1, CH	5.40, dd (9.9, 9.2)	69.5, CH	5.45, dd (9.9, 9.1)
7	126.8, CH	5.21, d (10.2)	127.1, CH	5.19, d (9.9)	127.0, CH	5.21, d (9.9)
8	139.4, qC		139.1, qC		139.3, qC	
9	38.9, CH_2	2.20, m 2.05, m	39.0, CH_2	2.25, m 2.10, m	39.0, CH_2	2.25, m 2.05, m
10	23.2, CH_2	2.20, m 2.10, m	23.3, CH_2	2.10, m	23.3, CH_2	2.30, m 2.10, m
11	124.4, CH	4.98, dd (5.9, 5.2)	124.50, CH	4.99, dd (5.4, 5.2)	124.5, CH	4.99, dd (5.2, 5.2)
12	133.6, qC		133.5, qC		133.5, qC	
13	36.7, CH_2	2.05, m 1.90, m	36.7, CH_2	2.05, 2H, m	36.7, CH_2	2.05, m 1.90, m
14	27.9, CH_2	1.65, m 1.30, m	27.9, CH_2	1.60, m 1.35, m	27.9, CH_2	1.65, m 1.25, m
15	33.0, CH	1.55, m	33.0, CH	1.55, m	33.0, CH	1.55, m
16	19.5, CH_3	0.77, d (6.6)	19.5, CH_3	0.77, d (6.6)	19.5, CH_3	0.77, d (6.6)
17	20.8, CH_3	0.80, d (6.6)	20.8, CH_3	0.80, d (6.6)	20.8, CH_3	0.81, d (6.6)
18	29.8, CH_3	1.38, s	29.8, CH_3	1.38, s	29.8, CH_3	1.39, s
19	16.3, CH_3	1.49, s	16.3, CH_3	1.49, s	16.9, CH_3	1.49, s
20	14.9, CH_3	1.72, s	14.9, CH_3	1.72, s	1490., CH_3	1.73, s
1′	155.7, qC		155.8, qC		156.0, qC	
2′	42.8, CH_2	3.59, brt (4.8)	36.0, CH_2	3.20, dt (7.0, 4.3)	45.2, CH_2	4.31, brs
3′	44.4, CH_2	3.50, brdt (5.9, 4.8)	15.3, CH_3	1.11, t (7.4)	138.5, qC	
4′, 8′					127.6, CH	7.26, 2H, m
5′, 7′					128.8, CH	7.32, 2H, m
6′					127.6, CH	7.27, d (8.4)
−NH		5.09 brt, (5.9)		4.60, brt (4.4)		4.95, brs

[a] In $CDCl_3$, 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR. Coupling constants (*J*) are in Hz. Carbon multiplicities were determined by DEPT135° or APT experiments. qC = quaternary, CH = methine, CH_2 = methylene, CH_3 = methyl carbons.

correlation with H_2-10 (δ 2.33 and 1.95) and a ^{2}J-HMBC correlation to C-10. The exomethylene protons H_2-20 showed a ^{3}J-HMBC correlation to C-11 (δ 56.8). The relative stereochemistry assignment at C-11 was aided by NOESY data. The proton doublet of doublets H-5a (δ 2.20) showed a strong NOESY correlation with H-6, suggesting its β-orientation. The α-oriented H-5b (δ 1.80) showed a strong NOESY correlation with H-10a (δ 2.33), which in turn showed a NOESY correlation with H-11, suggesting similar stereo-orientation. Therefore, compound **13** was identified as (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-2,7,12(20)-cembratriene-11-bromo-4,6-diol.

The HRESMS data of **14** suggested a molecular formula similar to that of **13** with the replacement of Br with Cl. The ^{1}H and ^{13}C NMR data of **14** (Tables 1 and 2) further supported this conclusion. The chlorinated carbon C-11 (δ 64.1) was assigned on the basis of its ^{3}J-HMBC correlation with H_2-20. The relative stereochemistry of C-11 was assigned in a similar fashion to that of **13**. Therefore, compound **14** was identified as (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*)-2,7,12(20)-cembratriene-11-chloro-4,6-diol.

Reflux of **1** in toluene with chlororethyl, ethyl, and benzyl isocyantes in the presence of a catalytic amount of triethylamine afforded the C-6 carbamates **15**−**17**, respectively.

The HRMS data analysis of **15** revealed the molecular formula $C_{23}H_{38}ClNO_3$ with the M and M + 2, 3:1, isotopic cluster characteristic pattern for a monochlorinated compound. The downfield shifting of the C-6 carbon chemical shift in **15** (+3.6 ppm) compared with that of the starting material **1** suggested carbamoylation at this position.[5,11] ^{1}H and ^{13}C NMR data (Table 3) further supported this fact. The carbonyl carbon at δ 155.7 was assigned C-1′. This was based on its ^{3}J-HMBC correlation with the methylene triplet H_2-2′ (δ 3.59), which in turn showed COSY coupling with the H_2-3′ doublet of triplets (δ 3.50).

The HRMS data of **16** suggested the molecular formula $C_{23}H_{39}NO_3$. The ^{1}H and ^{13}C NMR data (Table 3) were similar to those of **15**, with the replacement of the terminal chlorinated methylene C-3′ with a methyl group. The methyl triplet H_3-3′ (δ 1.11) was assigned on the basis of its COSY coupling with the H_2-2′ doublet of triplets (δ 3.20).

The ^{1}H and ^{13}C NMR data of **17** (Table 3) indicated a monosubstituted benzene moiety replacing the methyl C-3′ in **16**. The broad methylene singlet H_2-2′ (δ 4.31) showed a ^{3}J-HMBC correlation with the aromatic proton multiplet H-4′ and H-8′ (δ 7.26). The latter protons showed a COSY coupling with H-5′ and H-7′ multiplet (δ 7.32), which in turn showed a COSY coupling with the H-6′ doublet (δ 7.27).

Biocatalysis of **1** using *Mucor ramannianus* ATCC 9628 and *Cunninghamella elegans* ATCC 7929 afforded the known (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-2,7-cembradiene-11,12-epoxy-4,6-diol-6-*O*-acetate (**4**) as the main metabolite, an observation that was consistent with previous fermentation studies using plant cell cultures.[22] Biocatalysis of the cembranoid analogue (**3**) using the marine symbiotic *Bacillus megaterium* strain MO31, isolated form the Red Sea sponge *Negombata magnifica*, afforded the known (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*,10*R*)-2,7,11-cembratriene-4,6,10-triol (**18**) in 30% yield.[5,14]

Antiproliferative Activity. The effect of various concentrations of the cembranoids **1**−**18** was studied on the proliferation of the highly malignant mice +SA mammary epithelial cells.[25] Cembranoid **1** and analogues **6**, **7**, **11**, and **12** show potent antiproliferative activity against malignant +SA mammary epithelial cells at a 15–40 μM dose compared to their respective vehicle controls (Figure 1). This is a relevant activity compared with the positive drug control δ-tocotrienol, which showed IC_{50} 7 μM under the same assay conditions.[25] Anticancer activity was enhanced by the use of allylic oxidation at C-13 (Figure 1). Cembranoid **1** and its derivatives were found to be cytostatic, but not cytotoxic, to the neoplastic mammary epithelial cells grown in culture. The most active analogues were (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*,13*S*)-2,7,11-cembratriene-4,6,13-triol-6-*O*-acetate (**7**) and (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*,13*R*)-2,7,11-cembratriene-4,6,13-triol-6-*O*-acetate (**6**), with IC_{50} 20 and 30 μM, respectively (Figure 1). This clearly indicates that the C-13 hydroxy group enhances the binding affinity and the activity. The 13*S* configuration as in **7** was much more active than the 13*R*, suggesting that β-hydroxylation is more favorable for the activity. The microbial metabolite (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*,10*R*)-2,7,11-cembratriene-4,6,10-triol (**18**) had no affect on mammary tumor cell growth, indicating

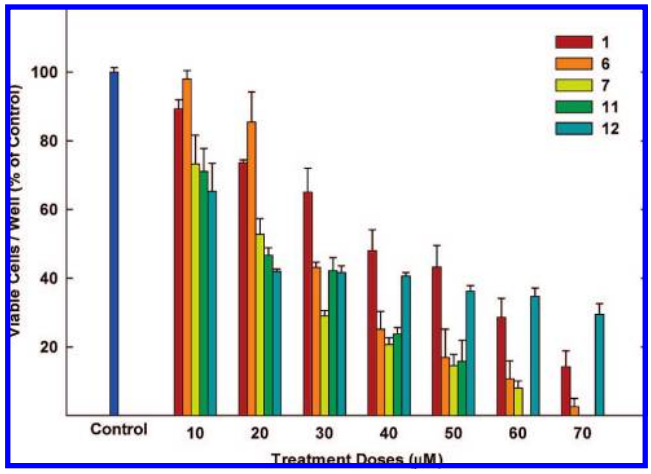


Figure 1. Effects of various doses of **1** and active analogues on malignant +SA mammary epithelial cell proliferation.

that C-10 hydroxylation reduces anticancer bioactivity, unlike C-13 hydroxylation.

The *cis* β-hydroxy thiocyanate (**8**) and the 1,3-oxathiolan-2-imine (**9**) analogues were devoid of activity, indicating that $\Delta^{11,12}$ is essential for activity and cannot be replaced with heterocyclic SP2, SP, or heteroatom-containing functional groups. The carbamates **15**–**17** were also inactive, suggesting the importance of the free C-6 hydroxy group. This is supported by the reduced activity of C-6-*O*-acetate (**3**), compared with **1**. Most likely **3** acts as a prodrug, which is activated by enzymatic acetate hydrolysis with the formation of free **1**. This conclusion was based on the rapid enzymatic acetate hydrolysis by most microbial species during biocatalytic screening, while **3** was stable for 14 days in the substrate control (tested compound in blank media without microorganisms).

Interestingly, although the α-ketol (1*S*,2*E*,4*S*,7*E*,11*E*)-2,7,11-cembratrien-4-ol-6-one (**10**) was almost devoid of antiproliferative activity, its geometrical isomer (1*S*,2*E*,4*S*,7*Z*,11*E*)-2,7,11-cembratrien-4-ol-6-one (**11**) was found to be a potent antiproliferative agent, suggesting the possible selectivity of certain double-bond geometry. Although this compound was no more active than its parent cembranoid (**1**) at higher doses, the secocembranoid **12** showed potent antiproliferative activity with an IC_{50} of 15 μM (Figure 1). This activity indicated that the 14-membered macrocycle is not essential for antiproliferative activity.

Experimental Section

General Experimental Procedures. The NMR data were acquired in $CDCl_3$, on a JEOL Eclipse NMR spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C. Optical rotations were measured on a Rudolph Research Analytical Autopol III polarimeter. IR spectra were recorded on a Varian 800 FT-IR Scimitar series. The HRESMS experiments were conducted at the University of Michigan on a Micromass LCT spectrometer. TLC analyses were carried out on precoated silica gel 60 F_{254} 500 μm TLC plates, using the developing system *n*-hexane/EtOAc (1:1) or $CHCl_3$/MeOH (9.5:0.5). For CC, Si gel 60 (particle size 63–200 μm) or Bakerbond octadecyl (C18), 40 μm, was used.

Extraction and Isolation. Fresh tobacco leaf powder (Custom Blends, New York, 27.2 kg) was extracted with *n*-hexane (45 L) in percolators three times at room temperature. The *n*-hexane extract was concentrated under vacuum, and the dried extract (1050 g) was vacuum liquid chromatographed on silica gel (200–300 mesh, 2 kg, Natland International Corporation) using a gradient of *n*-hexane/EtOAc to yield a crude cembranoid-containing fraction (64.0 g), which was further chromatographed on normal-phase and finally on reversed-phase silica gel (MeOH/H_2O, 2:3, isocratic) to give **1** (17.9 g) and **2** (3.6 g). The identity of **1** was confirmed by extensive NMR analysis and comparison with literature.[1,2,5,11]

Epoxidation of 1. To a solution of **2** (100 mg) in dioxane (3 mL) were added SeO_2 (20 mg) and 30% H_2O_2 (100 μL), and the solution was stirred at room temperature for 3 h. Water (10 mL) was then added, and the product was extracted with EtOAc. The EtOAc extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by CC on Si gel 60 (EtOAc/*n*-hexane, 2:3, isocratic) to give compounds **4** (42 mg, 42.2%) and **5** (20 mg, 20.1%).

Preparation of Compounds 6 and 7. To a solution of **3** (35.2 mg) in dioxane (2 mL) was added SeO_2 (11 mg), and the solution was stirred at room temperature for 5 h. Water (10 mL) was then added, and the product was extracted with EtOAc. The EtOAc extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by CC on Si gel 60 (EtOAc/*n*-hexane, 2:3, isocratic) to give compounds **6** (5.0 mg, 13.5%) and **7** (3.4 mg, 9.2%).

Preparation of Compounds 8 and 9. Compound **4** (34 mg) was dissolved in 5 mL of anhydrous CH_3CN. To this solution were added 19 mg of NH_4SCN and 10 mg of $SbCl_3$, and the mixture was stirred under reflux for 4 h. Water (10 mL) was then added, and the product was extracted with EtOAc. The EtOAc extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by CC (EtOAc/*n*-hexane, 2:3, isocratic) to give compounds **8** (3.7 mg, 9.4%) and **9** (5.4 mg, 13.7%).

Preparation of Compounds 10–12. Compound **1** (113.2 mg) was dissolved in 5 mL of pyridine. About 56.5 mg of CrO_3 was added, and the mixture was stirred at room temperature for 24 h. Water (10 mL) was then added, and the product was extracted with EtOAc. EtOAc extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **10** (84 mg). Compound **10** (84 mg) was dissolved in toluene (3 mL), and then 50 mg of NH_4SCN was added and the mixture was stirred with reflux for 5 h. Water (10 mL) was then added, and the product was extracted with EtOAc. The EtOAc extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by CC on Si gel (EtOAc/hexane, 2:3, isocratic) to give **11** (10.3 mg, 12.2%) and **12** (6.6 mg, 7.8%).

Preparation of 13 and 14. A solution of 25 mg of NBS or 36 mg of NCS in 1 mL of 10% aqueous acetone was slowly added to 2 mL of a solution of **1** (80 or 30.9 mg) in 10% aqueous acetone. The reaction mixture was stirred at room temperature for 15 min (NBS) or 18 h (NCS). Each reaction was stopped by adding water (10 mL), and the mixture was extracted with $CHCl_3$ (2 × 10 mL). The $CHCl_3$ layer was dried over anhydrous $NaSO_4$ and evaporated under vacuum to give a crude product. This crude product was chromatographed by CC on Si gel 60 using elution with *n*-hexane/EtOAc (1:1) to afford **13** (70.5 mg; 70%) or **14** (9.3 mg; 27%).

Preparation of Carbamates 15–17. To solutions of **1** (22.3, 73.3, or 88.8 mg) in toluene (2 mL) was added 59 μL of 2-chloroethyl isocyanate or 47 μL of ethyl isocyanate or 36 μL of benzyl isocyanate, respectively, and the solutions were separately mixed with 10 μL of Et_3N. Each solution was separately stirred and refluxed for 3 h. Water (10 mL) was then added, and the product of each reaction mixture was extracted with EtOAc. Each EtOAc extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Crude products were then purified by CC on Si gel 60 (EtOAc/*n*-hexane, 2:8 or 1:9) to give compounds **15**, 15.7 mg, 52%; **1d**, 49.6 mg, 55%; and **17**, 64.0 mg, 50%, respectively.

Symbiotic Bacteria Culture and Isolation. About 2 g of frozen *N. magnifica* voucher, which was collected and kept frozen in sterile bags, was macerated overnight in 0.5 L of Instant Ocean solution, and then the mixture was vacuum filter sterilized. Another 2 g of the sponge aseptically was blended in a sterile blender with 18 mL of the sponge Instant Ocean solution. Ten mL 1/10, $1/10^2$, $1/10^3$, $1/10^4$, and $1/10^5$ serial dilutions were made using the previously prepared Instant Ocean solution. About 100 μL of each concentration was inoculated on the top of sterile marine agar plates. Plates were incubated for 72 h at 28 °C. Symmetric fine colonies were separated and reinoculated on marine agar media. Pure cultures were subjected to PCR analysis, DNA extraction (MO Bio Laboratories, Inc., Carlsbad, CA), and finally 16S rRNA sequencing.[23] Obtained alignments were subjected to nucleotide blast queries. Identity was based on >99% matching.

Biocatalysis. Biocatalytic studies were conducted as described elsewhere.[24] Thirty growing fungal and marine bacterial cultures were used for screening of **1** and **3**.[24] *M. ramannianus* ATCC 9628 and *C. elegans* ATCC 7929 were selected for biocatalysis scale-up of **1**, while the marine symbiotic *B. megaterium* strain MO31 was selected for the scale-up of **3**. Each of these organisms was inoculated in ten 1000 mL

flasks each containing 250 mL of compound medium α (for fungi) or marine broth (for bacteria).[24] After 72 h, compounds **1** and **3** were added into their respective flasks (15 mg/flask). After 14 days, the growth medium was filtered and extracted with EtOAc (4 × 1000 mL). The EtOAc layer was then concentrated under vacuum. Residues obtained from biocatalysis of **1** or **3** were purified on a silica gel 60 column, followed by reversed-phase Si gel medium-pressure liquid chromatography (MPLC) to yield (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-2,7-cembradiene-11,12-epoxy-4,6-diol-6-*O*-acetate (**4**) as the main metabolite (38 mg, R_f 0.46, $CHCl_3$/MeOH, 9:1), along with other unstable minor metabolites. *B. megaterium* MO31 afforded the known (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*,10*R*)-2,7,11-cembratriene-4,6,10-triol (**18**) (46 mg, R_f 0.31, $CHCl_3$/MeOH, 9:1).

Compound 8: colorless oil; $[\alpha]^{25}_D$ +11.2 (*c* 0.26, $CHCl_3$); IR ν_{max} (neat) 3440, 2957, 2928, 2871, 2152, 1725, 1458 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2, respectively; HRESMS *m/z* 446.2341 $[M + Na]^+$ (calcd for $C_{23}H_{37}NO_4SNa$, 446.2341).

Compound 9: colorless oil; $[\alpha]^{25}_D$ +25.6 (*c* 0.22, $CHCl_3$); IR ν_{max} (neat) 3437, 2958, 2928, 2856, 1727, 1602, 1485 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2, respectively; HRESMS *m/z* 446.2343 $[M + Na]^+$ (calcd for $C_{23}H_{37}NO_4SNa$, 446.2341).

Compound 11: colorless oil; $[\alpha]^{25}_D$ +57.8 (*c* 0.69, $CHCl_3$); IR ν_{max} (neat) 3455, 2957, 2928, 2871, 1669, 1606 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2, respectively; HRESMS *m/z* 327.2288 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_2Na$, 327.2300).

Compound 13: yellowish oil; $[\alpha]^{25}_D$ +27.8 (*c* 0.47, $CHCl_3$); IR ν_{max} (neat) 3482, 2955, 29298, 2870, 16429, 1457 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2, respectively; HRESMS *m/z* 407.1564 $[M + Na]^+$ (calcd for $C_{20}H_{33}BrO_2Na$, 407.1562).

Compound 14: yellowish oil; $[\alpha]^{25}_D$ +43.8 (*c* 0.062, $CHCl_3$); IR ν_{max} (neat) 3502, 2956, 2927, 2856, 1602, 1462 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2, respectively; HRESMS *m/z* 363.2049 $[M + Na]^+$ (calcd for $C_{20}H_{33}ClO_2Na$, 363.2067).

Compound 15: colorless oil; $[\alpha]^{25}_D$ +59.8 (*c* 0.54, $CHCl_3$); IR ν_{max} (neat) 3450, 2995, 2928, 1713, 1605, 1509, 1457, 1371, 1305, 1258 cm^{-1}; ^1H and ^{13}C NMR see Table 3; HRESMS *m/z* 434.2433 (calcd for $C_{23}H_{38}ClNO_3Na$, 434.2438).

Compound 16: colorless oil; $[\alpha]^{25}_D$ +54.1 (*c* 0.10, $CHCl_3$); IR ν_{max} (neat) 3452, 2928, 2855, 1711, 1508, 1459, 1380, 1139, 1099 cm^{-1}; ^1H and ^{13}C NMR see Table 3; HRESMS *m/z* 400.2838 (calcd for $C_{23}H_{39}NO_3Na$, 400.2828).

Compound 17: yellowish oil; $[\alpha]^{25}_D$ +56.4 (*c* 0.41, $CHCl_3$); IR ν_{max} (neat) 3450, 2995, 2928, 1714, 1603, 1509, 1455, 1366, 1261, 1128 cm^{-1}; ^1H and ^{13}C NMR see Table 3; HRESMS *m/z* 462.2973 $[M + Na]^+$ (calcd for $C_{28}H_{41}NO_3Na$ a, 462.2984).

Antiproliferative Assay. The antiproliferative effects of semisynthetic derivatives were tested in culture on the highly malignant +SA mouse mammary epithelial cell line maintained on serum-free media and containing 10 ng/mL EGF and 10 μg/mL insulin as mitogens, as described previously in detail.[25] Cells were plated at a density of 5×10^4 cells/well (6 wells/group) in 24-well culture plates and fed media containing various concentrations (0.01–1000 μM) of each compound. After a 4-day culture period, viable +SA cell number was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay as described previously.[25]

Acknowledgment. This publication was made possible by the support of Philip Morris USA Inc. and Philip Morris International as well as the NIH grant no. P20RR16456 from the BRIN Program of the National Center for Research Resources. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. The American Foundation for Pharmaceutical Education is acknowledged for fellowship support to J.A.P.

References and Notes

(1) Wahlberg, I.; Enzell, C. R. *Beitr. Tabakforsch. Int.* **1984**, *12*, 93–104.
(2) Wahlberg, I.; Eklund, A. M. *Trends in Flavour Research*; Maarse, H.; van der, Heji, Eds.; Elsevier Science: Oxford, UK, 1994; pp 449–462.
(3) Eklund, A.-M.; Hatanaka, S.-I.; Wahlberg, I. *Progress in the Chemistry of Organic Natural Products*; Springer-Verlag Wien: New York, 1992; pp 143–293.
(4) Dauben, W. G.; Thiessen, W. E.; Resnick, P. R. *J. Am. Chem. Soc.* **1962**, *84*, 2015–2016.
(5) Wahlberg, I.; Olsson, E.; Berg, J. E. *Progress in Flavour Precursor Studies Proceedings of International Conference*; Schreier, P., Winterhalter, P., Eds.; Allured Publishing Corporation: Carol Stream, IL, 1993; pp 83–95.
(6) Ferchmin, P. A.; Hao, J.; Perez, D.; Penzo, M.; Maldonado, H. M.; Gonzalez, M. T.; Rodriguez, A. D.; Jean, D. V. *J. Neurosci. Res.* **2005**, *82*, 631–641.
(7) Olsson, E.; Holth, A.; Kumlin, E.; Bohlin, L.; Wahlberg, I. *Planta Med.* **1993**, *59*, 293–295.
(8) Saito, Y.; Takayawa, H.; Konishi, S.; Yoshida, D.; Mizusaki, S. *Carcinogenesis* **1985**, *6*, 1189–1194.
(9) Saito, Y.; Nishino, H.; Yoshida, D.; Mizusaki, S.; Ohnishi, A. *Oncology* **1988**, *45*, 122–126.
(10) Saito, Y.; Tsujino, Y.; Kaneko, H.; Yoshida, D.; Mizusaki, S. *Agric. Biol. Chem.* **1987**, *51*, 941–943.
(11) Wahlberg, I.; Eklund, A. M. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, C. H., Eds.; Springer-Verlag, Wien: New York, 1992; Vol. 59, pp 142–293.
(12) Sawant, S.; Youssef, D.; Mayer, A.; Sylvester, P.; Wali, V.; Arant, M.; El Sayed, K. *Chem. Pharm. Bull.* **2006**, *58*, 1119–1123.
(13) Behr, D.; Wahlberg, I.; Nishida, T.; Enzell, C. R.; Berg, J. E.; Pilotti, A. M. *Acta Chem. Scand. Ser. B: Org. Chem. Biochem.* **1980**, *B34*, 195–202.
(14) Forsblom, I.; Berg, J. E.; Wahlberg, I. *Acta Chem. Scand.* **1993**, *47*, 80–88.
(15) (a) Van Tamelen, E. E. *J. Am. Chem. Soc.* **1951**, *73*, 3444–3448. (b) Sharghi, H.; Nasseri, M. A.; Niknam, K. *J. Org. Chem.* **2001**, *66*, 7287–7293.
(16) Tamami, B.; Mahdavi, H. *Tetrahedron Lett.* **2002**, *43*, 6225–6228.
(17) Yadav, J. S.; Reddy, B. V. S.; Reddy, S. *Tetrahedron Lett.* **2004**, *45*, 1291–1293.
(18) Akhrem, A. A.; Istomina, Z. I.; Turuta, A. M.; Kogan, G. A. *B. Acad. Sci. USSR CH+* **1973**, *22*, 82–87.
(19) Kabas, G. *J. Org. Chem.* **1967**, *32*, 218–220.
(20) Iwagawa, T.; Nakamura, S.; Okamura, H.; Nakatami, M. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 3543–3549.
(21) Wahlberg, I.; Forsblom, I.; Vogt, C.; Eklund, A.-M.; Nishida, T.; Enzell, C. R.; Berg, J. E. *J. Org. Chem.* **1985**, *5*, 4527–4538.
(22) El Sayed, K. A.; Sylvester, P. W. *Expt. Opin. Invest. Drugs* **2007**, *16*, 877–887.
(23) (a) Weisburg, W. G.; Barns, S. M.; Pelletier, D. A.; Lane, D. J. *J. Bacteriol.* **1991**, *173*, 697–703. (b) Reysenbach, A.-L.; Giver, L. J.; Wickham, G. S.; Pace, N. R. *Appl. Environ. Microbiol.* **1992**, *58*, 3417–3418.
(24) El Sayed, K. A.; Hamann, M. T.; Waddling, C. A.; Jensen, C.; Lee, S. K.; Dunstan, C. A.; Pezzuto, J. M. *J. Org. Chem.* **1998**, *63*, 7449–7455.
(25) McIntyre, B. S.; Briski, K. P.; Gapor, A.; Sylvester, P. W. *PSEBM* **2000**, *224*, 292–301.

NP0704351