Topsentin, Bromotopsentin, and Dihydrodeoxybromotopsentin: Antiviral and Antitumor Bis(indolyl)imidazoles from Caribbean Deep-Sea Sponges of the Family Halichondriidae. Structural and Synthetic Studies[1]

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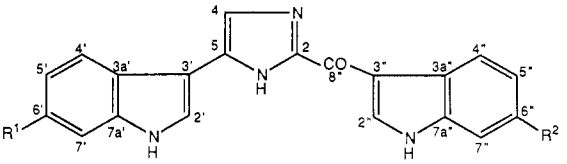
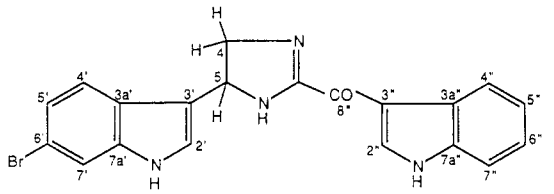
Isolation and structure elucidation of topsentin (1) and bromotopsentin (2) are described. These novel indole alkaloids have been shown to have antitumor and antiviral activities. Their structures were determined on the basis of spectral data, and bromotopsentin was correlated structurally with topsentin by catalytic debromination. Syntheses of 1 and several analogues are also reported.

As an outgrowth of our search for biologically active compounds from marine organisms, we report here that extracts of four related Caribbean deep-sea sponges[3] collected by Johnson-Sea-Link submersible of the Harbor Branch Oceanographic Institution (HBOI) (at Chub Cay, Bahamas, at -174 m, and at Goulding's Cay, Bahamas, at -229 and -355 m) inhibit the growth of P388 mouse leukemia cells and Herpes simplex virus, type 1 (HSV-1). These activities were found by TLC bioautography (P388 and HSV-1) to be associated with two bright-yellow bis(indolyl)imidazoles, topsentin (1) and bromotopsentin (2), and with the related dihydro compound 3. Their structures represent a novel variation on bis(indoles) previously reported from marine sources.[4-6] In addition, we describe the syntheses of topsentin (1) and its analogues 4-9.

Bartik et al.[7] have independently recently reported the isolation of topsentin (1), bromotopsentin (2), and deoxytopsentin (6), which they called topsentins-B1, -B2, and -A, respectively, from a Mediterranean shallow-water sponge, *Topsentia genitrix*. Similarly Braekman et al. have reported the synthesis of 6[8] but by a route quite different from that reported here. These authors indicated that topsentins are toxic to fish but did not ascribe antiviral or antitumor properties to the compounds.[9]



1: R^1 = H, R^2 = OH (Topsentin)
2: R^1 = Br, R^2 = OH (Bromotopsentin)
4: R^1 = OH, R^2 = H (Isotopsentin)
5: R^1 = R^2 = OH (Hydroxytopsentin)
6: R^1 = R^2 = H (Deoxytopsentin)



3 (4,5-Dihydro-6"-deoxybromotopsentin)

Isolation and Structure Assignments of Topsentin (1) and Bromotopsentin (2). Frozen sponge specimens were homogenized in methanol or methanol-toluene, and the extracts were concentrated and partitioned between organic and aqueous phases. The organic phase, on silica gel column chromatography with chloroform-methanol followed by reversed-phase high-performance liquid chromatography (RP-HPLC) with aqueous methanol, gave topsentin (1) and bromotopsentin (2). Typical yields were 0.1% of 1 and 0.3% of 2 (wet weight).

Topsentin had the molecular formula $C_{20}H_{14}N_4O_2$ and bromotopsentin the formula $C_{20}H_{13}BrN_4O_2$ by HREIMS. The suspicion that 2 was a bromo derivative of 1 was confirmed by hydrogenolysis of 2 to give 1 in quantitative yield.

The ^1H NMR spectra of 1 and 2 in deuteriated methanol, acetone, or dimethyl sulfoxide were complicated by

(1) (a) Presented in part at the 194th National Meeting of the American Chemical Society, New Orleans, LA, Aug 30-Sept 4, 1987; S. P. Gunasekera, Y. Kashman, Abstract ORGN 276. (b) Patent applications have been filed for topsentin (SP10058) and bromotopsentin (SP10059) (November 22, 1986), as well as for dihydrodeoxybromotopsentin (SP10095; July 17, 1987).

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(3) The Order Halichondrida (Phylum Porifera, Class Demospongiae) is a problematic taxonomic group, with generic distinctions not clearly defined. The four samples examined in this study have been assigned to the genus *Spongosorites*, Topsent 1896 (see the Experimental Section), a genus characterized by a distinct and thick (up to 1 mm) dermal layer of smaller spicules arranged tangentially to the surface; a confused choanosomal arrangement of spicules with sporadic vague spicule tracts running parallel to the surface; bright-yellow color when alive, turning brown or black when preserved in alcohol; and two or three size categories of straight or crooked oxea. *Spongosorites* sp. 1 (4-XII-84-1-22, black in alcohol) has crooked oxea and is distinguished by association with vermetids (Phylum Mollusca, Class Gastropoda); *Spongosorites* sp. 3 (4-XII-84-1-23 and 23-VIII-85-1-39, tan-brown in alcohol) has fusiform straight oxea. Voucher specimens are deposited in the Indian River Coastal Zone Museum of the HBOI, and species names will be assigned when revision of the Order Halichondrida has been completed by Dr. R. W. M. Van Soest, Institute for Taxonomic Zoology, University of Amsterdam, with S.A.P. and M.C.D.

(4) Norton, R. S.; Wells, R. J. *J. Am. Chem. Soc.* **1982**, *104*, 3628-3635.

(5) Moquin, C.; Guyot, M. *Tetrahedron Lett.* **1984**, *25*, 5047-5048.

(6) Kohmoto, S.; Kashman, Y.; McConnell, O. J.; Rinehart, K. L.; Wright, A.; Koehn, F. E. *J. Org. Chem.*, in press.

(7) Bartik, K.; Braekman, J.-C.; Daloze, D.; Stoller, C.; Huysecom, J.; Vandevyver, G.; Ottinger, R. *Can. J. Chem.* **1987**, *65*, 2118-2121.

(8) Braekman, J. C.; Daloze, D.; Stoller, C. *Bull. Soc. Chim. Belg.* **1987**, *96*, 809-812.

(9) Compounds in this series have also very recently been identified in extracts of a British Columbia deep-water sponge tentatively identified as an *Hexadella* species (Andersen, R. J., 16th International Symposium on the Chemistry of Natural Products, IUPAC, Kyoto, May 29-June 3, 1988).

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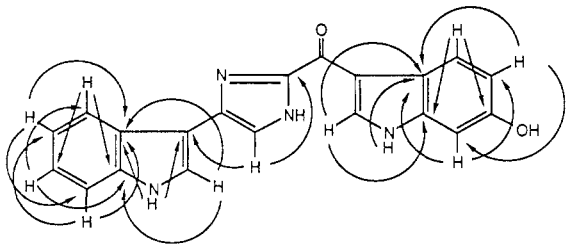
Scheme I. Results of Long-Range (H-C-C-C) ^{13}C-^{1}H Correlations for 1 Employing J_{CH} = 8 Hz and C-H Correlation by XCHCORR[14]



concentration and pH dependence and broadening or doubling of lines. The ^{13}C NMR spectra of 1 and 2 were also solvent-dependent, showing for example only 17 resonances in deuteriomethanol. Optimal ^{1}H NMR data were obtained in acidic mixtures ($CDCl_3$–CF_3COOH or Me_2SO-d_6–CF_3COOH); the complicated spectra were simplified, and all 20 carbon atoms were observed. Spectra obtained are summarized in Table I; assignments were confirmed by ^{1}H–^{1}H and ^{13}C–^{1}H (direct, long-range) correlated spectral data and by DEPT data.

The ^{1}H NMR spectrum of 1 contains a four-proton set (H-4′ to H-7′) appropriate for an indole unsubstituted in the benzene ring (Table I),[10] while that of 2 contains a three-proton set (H-4′, H-5′, H-7′) for a similar but bromine-substituted unit;[10] spectra of both 1 and 2 contain a three-proton set (H-4″, H-5″, H-7″) for an indole with hydroxyl substitution in the benzene ring (Table I.)[11] There are two protons (H-2′, H-2″) with chemical shifts and coupling constants appropriate for indole α-hydrogens (Table I);[10] for example, the ^{13}C–^{1}H coupling constants are 187 Hz (δ 8.07; H-2′ for 1) and 188 Hz (δ 8.74; H-2″),[12] and each proton is coupled to an indole NH proton. The UV spectra of 1 and 2 (cf. the Experimental Section) are appropriate for indoles conjugated to other chromophores.[10] A major fragment ion (C_8H_7NO) in the mass spectra of both 1 and 2 represents the hydroxyindole unit, while a $C_9H_6NO_2$ fragment ion (1 and 2) and a cross-conjugated ketonic carbon (δ_{13} 173.6 for 1, 175.3 for 2) extend that unit to a hydroxyindole-3-carbonyl unit.

Location of the bromine and hydroxyl groups at C-6′ and C-6″, respectively, was suggested by comparison of the chemical shifts of the protons on the benzene rings with those of known bromo- and hydroxyindoles,[10,11,13] and confirmed by extensive ^{13}C–^{1}H correlations, presented in two ways, in Scheme I (for 1) and Table II (for 2).

The indole and hydroxyindole carbonyl units together account for $C_{17}H_{12}N_2O_2$ of the topsentin formula, leaving $C_3H_2N_2$ unassigned. The missing unit could be either a 3,4-disubstituted pyrazole or a 2,4-disubstituted imidazole ring, but the latter fits better a biosynthetic pathway based on condensation of two tryptamine precursors. This postulate was confirmed by NMR data for C-4 and H-4 in 1 (δ_{13} 118.0,[18] δ_H 7.96, J_{C-H} = 201 Hz),[12] and by ^{13}C–^{1}H correlations (Scheme I). For example, in the long-range ^{13}C–^{1}H correlated NMR spectrum (enhanced $^{3}J_{C-H}$ = 8 Hz) a correlation between C-3 and H-4 was observed. Finally, the imidazole and other structural features were confirmed by synthesis of 1, as described next.

Synthesis of Topsentin. As a confirmation of the structure (1) assigned to topsentin, a total synthesis of the natural product was undertaken. It was recognized above that the molecule is pseudosymmetrical and is presumably formed biosynthetically from two tryptamine equivalents. The synthon chosen in the synthesis to represent tryptamine was glyoxalylindole, which could condense with an ammonia equivalent to give the desired unsymmetrical imidazole (together with three other products) if a mixture of the appropriate indoles were chosen or a single imidazole if a single indole were employed. (The isolation of a single topsentin argues, incidentally, for an enzymatic biosynthesis.)

Two approaches to the key glyoxalylindoles 14 and 15 were evaluated. In one approach (Scheme II) the 3-(chloroacetyl)indoles 10 and 11[19] were hydrolyzed to (hydroxyacetyl)indoles by heating in formamide–water (10:1) (12, 97% from 10; 13, 82% from 11) and then 12 and 13 were oxidized in situ to the requisite glyoxalylindoles 14 and 15; the latter were condensed with ammonia to the imidazoles. Treatment of a 4:1 mixture of 3-(hydroxyacetyl)indole (12) and 3-(hydroxyacetyl)-6-(benzyloxy)-indole (13) with copper(II) acetate in the presence of ammonia[20] produced a mixture of *O*-benzyltopsentin (16, 9%, 15% based on recovered 13), *O*-benzylisotopsentin (17, 7%, 12% based on recovered 13), *O*,*O*′-dibenzyl-6′-hydroxytopsentin (18, 3%, 5% based on recovered 13), and deoxytopsentin (6, 17%, 31% based on recovered 12). The individual products were then separated and (where necessary) debenzylated in quantitative yield by hydrogenolysis over 10% palladium on charcoal to give the parent compounds: topsentin (1), isotopsentin (4), hydroxytopsentin (5). The synthesized topsentin was identical with natural topsentin in all spectral data and biological activities.

By an alternative route (Scheme II), the oxidation of the (hydroxyacetyl)indoles was carried out separately, and the isolated glyoxalylindoles 14 and 15 were then combined with ammonia to give the same mixture of 16–18 and 6.

Of the two routes, the overall yield from the (hydroxyacetyl)indoles was somewhat higher when the glyoxalylindoles were isolated, then combined, though this involved an extra step. This route (involving isolation of 15) was also employed in an improved preparation of 18 in 63% yield.

Isolation of 4,5-Dihydro-6″-deoxybromotopsentin (3). Compound 3 was isolated in small amounts (0.005% vs 0.1% of 2 from a sponge sample tentatively identified as *Spongosorites* sp. 3) as an amorphous yellow powder, following repeated chromatography of the combined methanol extracts of the sponge. The UV spectrum of 3 (cf. the Experimental Section) is appropriate for an indole conjugated to other chromophores,[10] and intense IR absorptions were found at 3620, 3390 (OH and/or NH), and 1665 cm^{-1} (carbonyl).

The ^{1}H NMR spectrum of 3 (Me_2SO-d_6, Table I) argued for unsubstituted and monosubstituted benzene rings in the indoles, while the two pyrrole ring protons (H-2′, H-2″) were observed at δ 7.29 and 8.38 (both br s), with the former well upfield from 1 and 2 and the chemical shift

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(16) Same as XHCORR with suitable delay values.
(17) Kessler, H.; Griesinger, C.; Zarbock, J.; Loosli, H. R. *J. Magn. Reson.* **1984**, *57*, 331–336.
(18) Reference 12, p C135.
(19) Bergman, J. *J. Heterocycl. Chem.* **1970**, *7*, 1071–1076.
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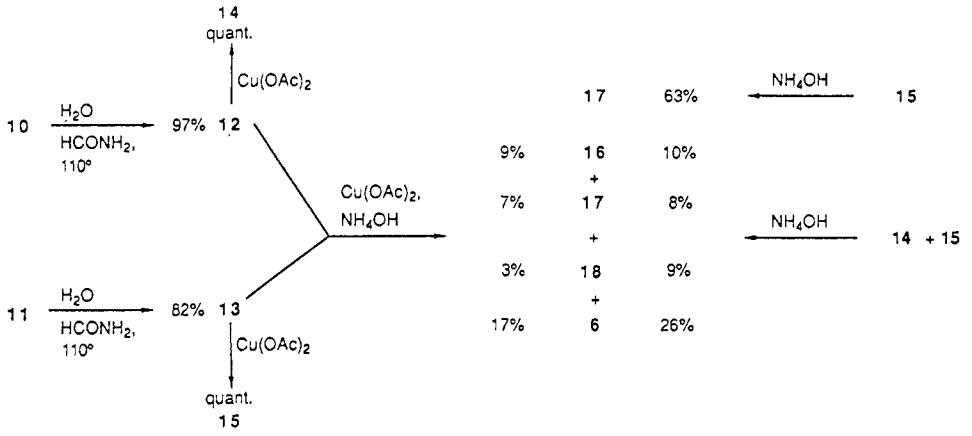
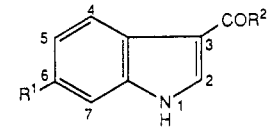
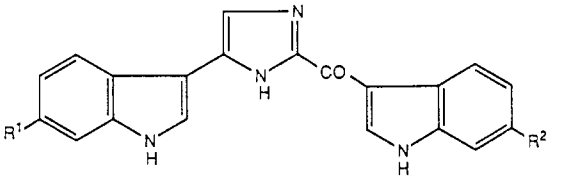
Scheme II. Synthetic Routes to Topsentin Derivatives







10: R^1 = H, R^2 = $-CH_2Cl$

11: R^1 = –OBzl, R^2 = $-CH_2Cl$

12: R^1 = H, R^2 = $-CH_2OH$

13: R^1 = –OBzl, R^2 = $-CH_2OH$

14: R^1 = H, R^2 = –CHO

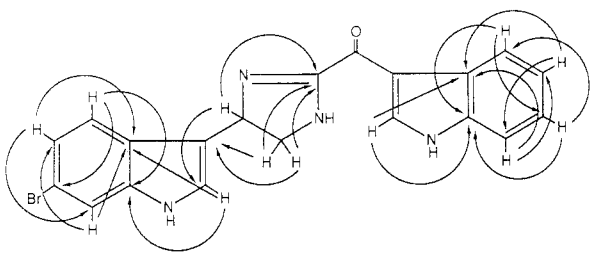
15: R^1 = –OBzl, R^2 = –CHO

16: R^1 = H, R^2 = –OBzl

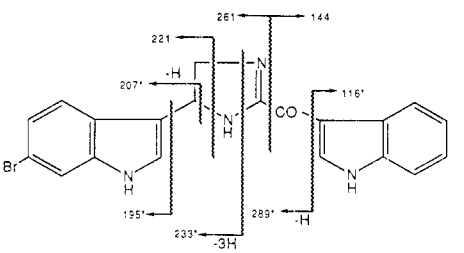
17: R^1 = –OBzl, R^2 = H

18: R^1 = R^2 = –OBzl

Scheme III. Results of Long-Range (H–C–C–C) ^{13}C–^1H Correlation for 3, Employing J_{CH} = 8 Hz



Scheme IV. MS Fragmentations of 3[a]



[a] Values marked by an asterisk (*) were confirmed by HREIMS.

difference arguing the latter to be on a pyrrole ring with additional conjugation (to a carbonyl group). In partial confirmation of these structures, J_{CH} values of 183 and 188 Hz measured for C-2′ and C-2″ confirmed their proximity to nitrogen atoms.[12] The ^1H NMR spectrum also showed signals at δ 11.52, 11.13, and 8.47 (all br s) for three slowly exchanging NH protons. Most significant were the signals observed for a deshielded ABX system (A, δ 3.60, ddd, J = 12.1, 4.6, and 4.4 Hz; B, δ 3.45, ddd, J = 12.1, 9.5, and 2.1 Hz; X, δ 5.23, dd, J = 9.5 and 4.7 Hz), NCH_2CHN. The homonuclear COSY spectrum placed the methine of this $>CHCH_2$ unit at the 3-position of the disubstituted indole (Scheme III), where the chemical shift of the methine proton (far downfield, at δ 5.23) is appropriate for double deshielding—by the indole and one of the nitrogens—vide infra. A nitrogen also has to be attached to the deshielded methylene unit (δ_H 3.60, 3.45).

The ^{13}C NMR spectrum of **3** exhibited 18 sp^2 and two sp^3 carbon signals (Table I). The chemical shifts of the protons and carbons, together with a homonuclear (^1H) COSY experiment, suggested 3-substituted and 3,6-disubstituted indole systems. Completion of the structure **3** requires insertion of two more carbon atoms (δ 159.1, 160.5) and an oxygen atom, including a carbonyl group (IR 1665 cm^{-1}). The carbonyl carbon (C-8″, δ 159.1) is found at relatively high field even for a cross-conjugated carbonyl, which might be explained by the nitrogen atoms in this system (a conjugated amide equivalent). The suggested ring systems were unequivocally confirmed by XHCORR,[14] XHCORRLR,[16] COLOC,[17] and HETCOSY[15] experiments as summarized in Scheme III. Long-range correlations between H-4 and C-2′ and between H-5 and C-2 are particularly important for the suggested structure.

The HREI and LRFD mass spectra did not contain a molecular ion; rather, the highest peak observed was at m/z 404.0300 (M – 2H, Δ 2.8 mmu for $C_{20}H_{13}{}^{79}BrN_4O$), resulting from loss of two protons to give an aromatic ring. Support for the suggested structure came from the fragments observed in the mass spectrum (Scheme IV), where bromine label unequivocally differentiated between the 3,6-disubstituted indole and the 3-acylindole, and high-resolution measurements confirmed most of the ionic compositions. The carbonyl (C-8″) was located by the fragmentations to give m/z 144 and 289, and the fragmentation of the C-4, C-5 bond (between two sp^3 carbons) to give m/z 207 contrasted with the absence of such fragmentation in **1** and **2**.

Table I. ^1H and ^{13}C NMR Data for Topsentins: Chemical Shift, ppm, from Me_2SO (Multiplicity; J, Hz)[a]

assign.,	1		2		3				6				
C or H	δ_H[b]	δ_C[c]	δ_H[b]	δ_C[c]	δ_H[d]	δ_C[e]	4, δ_H[f]	5, δ_H[f]	δ_H[b]	δ_C[c]	16, δ_H[f]	17, δ_H[f]	18, δ_H[f]
2		143.09		144.90		160.49				143.94			
3					8.47 (br s)								
4	7.96 (s)	118.04	7.85 (s)	119.33 (d, 201)	3.60 (ddd, 12.1, 4.6, 4.4)	45.08 (t, 137)	8.01 (s)	8.08 (s)	7.85 (s)	119.30 (d, 198)	8.01 (s)	7.99 (s)	8.06 (s)
					3.45 (ddd, 12.1, 9.5, 2.1)								
5		132.59		133.75	5.23 (dd, 9.5, 4.7)	55.55 (d, 136)				133.33			
1′ (N)	11.64 (d, 2.6)		11.64 (d, 2.6)		11.13 (br s)		11.24 (br s)	11.30 (br s)	11.53 (d, 2.6)		11.62 (br s)	11.41 (br s)	11.44 (br s)
2′	8.07 (d, 2.6)	124.95 (d, 187)[g]	8.06 (d, 2.6)	125.14 (d, 189)[c]	7.29 (br s)	124.67 (d, 183)	7.87 (d, 3.2)	7.90 (d, 2.1)	8.07 (d, 2.6)	124.23 (d, 187)	8.08 (d, 1.8)	7.95 (d, 1.8)	7.96 (d, 1.8)
3′		104.91		107.42		116.18				105.98			
3a′		124.68		124.03		126.45				124.61			
4′	8.02 (d, 7.0)	119.83 (d, 158)	8.02 (d, 8.4)	121.85 (d, 162)	7.67 (d, 8.5)	121.49 (d, 159)	7.75 (d, 8.3)	7.76 (d, 8.7)	8.05 (dd, 1.5, 6.9)	119.77 (d, 157)	8.03 (br d, 7)	7.89 (d, 8.8)	7.89 (d, 8.8)
5′	7.19 (m)	120.33 (d, 158)	7.29 (dd, 2.0, 8.4)	123.00 (d, 167)	7.12 (dd, 8.5, 1.7)	123.05 (d, 165)	6.67 (dd, 1.8, 8.3)	6.70 (dd, 1.8, 8.7)	7.18 (m)	119.94 (d, 158)	7.19 (m)	6.89 (dd, 1.8, 8.8)	6.90 (dd, 1.8, 8.8)
6′	7.19 (m)	122.28 (d, 157)		114. 93		116.04			7.18 (m)	121.89 (d, 158)	7.19 (m)		
7′	7.50 (br d, 7)	112.36 (d, 158)	7.70 (d, 2.0)	114.93 (d, 167)	7.58 (d, 1.7)	115.36 (d, 165)	6.80 (d, 1.8)	6.83 (d, 1.8)	7.50 (dd, 1.5, 6.9)	112.05 (d, 158)	7.49 (br d, 7)	7.06 (d, 1.8)	7.06 (d, 1.8)
7a′		136.74		137.74		139.34				136.46			
1″ (N)	12.11 (d, 3.0)		11.96 (d, 2.9)		11.52 (br s)		12.51 (d, 2.7)	12.25 (d, 2.7)	12.33 (d, 3.0)		12.29 (br s)	12.46 (br s)	12.33 (br s)
2″	8.74 (d, 3.0)	137.06 (d, 188)	8.92 (d, 2.9)	136.62 (d, 192)	8.38 (br s)	132.94 (d, 188)	8.72 (d, 2.7)	8.47 (d, 2.7)	9.15 (d, 3.0)	137.49 (d, 189)	8.74 (d, 2.4)	8.86 (d, 2.4)	8.63 (d, 2.4)
3″		114.16		114.32		112.72				113.76			
3a″		119.30		119.85		127.55				126.56			
4″	8.10 (d, 8.4)	122.28 (d, 163)	8.16 (d, 8.3)	122.58 (d, 163)	8.37 (ddd, 8.0, 1.0, 0.7)	123.60 (d, 158)	8.26 (dd, 3.6, 7.8)	8.02 (d, 8.4)	8.41 (dd, 3.4, 5.8)	121.59 (d, 158)	8.17 (d, 8.8)	8.31 (dd, 2.7, 4.5)	8.14 (d, 8.8)
5″	6.81 (dd, 1.8, 8.6)	112.84 (d, 157)	6.82 (dd, 2.4, 8.3)	112.74 (d, 157)	7.02 (ddd, 8.0, 8.0, 1.0)	121.93 (d, 158)	7.29 (m)	6.81 (dd, 1.8, 8.4)	7.29 (m)	122.28 (d, 159)	7.02 (dd, 1.8, 8.8)	7.31 (m)	7.03 (dd, 1.8, 8.8)
6″		155.02		154.84	7.13 (ddd, 8.0, 8.0, 1.0)	123.75 (d, 158)	7.29 (m)		7.29 (m)	123.31 (d, 157)		7.31 (m)	
7″	6.93 (d, 1.8)	98.02 (d, 157)	6.96 (d, 2.4)	98.07 (d, 160)	7.42 (ddd, 8.0, 1.0, 0.7)	112.36 (d, 158)	7.56 (m)	6.92 (d, 1.8)	7.59 (dd, 3.2, 5.9)	112.55 (d, 158)	7.14 (d, 1.8)	7.58 (dd, 1.5, 5.1)	7.14 (d, 1.8)
7a″		138.16		138.04		138.06				136.54			
8″		173.58		175.32		159.12				174.85			
OCH_2												5.15 (s, 2 H)	5.16 (s, 2 H)
OCH_2											5.17 (s, 2 H)		5.17 (s, 2 H)
C_5H_5											7.3–7.6 (m, 5 H)	7.3–7.6 (m, 5 H)	7.3–7.6 (m, 5 H)

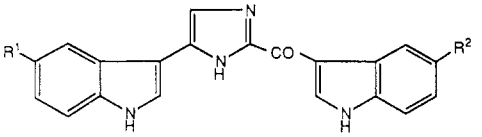
[a] 1% TFA in Me_2SO-d_6, except as noted; s = singlet, d = doublet, m = multiplet, t = triplet, br = broad. [b] 200 MHz. [c] 75 MHz. [d] 360 MHz. [e] 90.5 MHz, MeOH-d_4. [f] 300 MHz. [g] Bruker's coupled DEPT pulse sequence was used.

Table II. ^{13}C–^{1}H Correlations for 2[a–c]

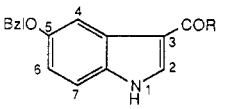
^{13}C	^{1}H 5	1′	2′	4′	5′	7′	1″	2″	4″	5″	7″
2	▲										
4	●										
5	▲										
	○										
2′		▲	▲△								
			○								
3′		▲		●							
		●									
3a′		▲△	▲△		▲△	▲△					
			●		●	●					
4′				○							
5′					▲	▲					
					○	●					
6′				▲△							
				●							
7′					▲△						
					●						
7a′			▲	▲△							
			●	●							
2″								▲△			
								○			
3″							▲				
							●				
3a″							▲△	▲△		▲△	▲△
							●	●		●	●
4″									▲△		
									○		
5″										▲△	▲△
										○	●
6″									▲△		
									●		
7″										▲△	▲△
										●	○
7a″								▲△	▲△		
								●	●		

[a] $CDCl_3$–TFA, 1:1. [b] δ values reported in Table I. [c] ▲, HETCOSY;[15] ○, XHCORR;[14] ●, XHCORRLR;[16] △, COLOC.[17]

Syntheses of Neotopsentin and Related Compounds. To investigate further the structure–activity relationship, we prepared the 5″-analogue of topsentin, which we have named neotopsentin (**7**), from 5″-(benzyloxy)indole via the isolated glyoxalylindole **21**. This preparation yielded a mixture of the *O*-benzyl products **22**–**24** as well as deoxytopsentin (**6**) in yields of 11, 9, 3, and 28%, respectively. The *O*-benzyl products were then converted quantitatively to **7**, neoisotopsentin (**8**), and neohydroxytopsentin (**9**). Again, a more efficient preparation of **9** involved treatment of **21** alone with ammonia (yield of **24**, 60%).



7: R^1 = H, R^2 = OH (Neotopsentin)
8: R^1 = OH, R^2 = H (Neoisotopsentin)
9: R^1 = R^2 = OH (Neohydroxytopsentin)
22: R^1 = H, R^2 = –OBzl
23: R^1 = –OBzl, R^2 = H
24: R^1 = R^2 = –OBzl



19: R = $-CH_2Cl$
20: R = $-CH_2OH$
21: R = –CHO

Biological Activity. All of the topsentins and their analogues reported here are active as antiviral and cytotoxic (antitumor) agents.[1b] Compounds **1** and **2** had in vitro activity against HSV-1, *Vesicular stomatitis* virus (VSV), and the corona virus A-59. Compound **1** had in vitro activity against P388 (IC_{50} 3.0 μg/mL) and human tumor cells (HCT-8, A-549, T47D, 20 μg/mL) and in vivo activity against P388 (T/C 137%, 150 mg/kg) and B16 melanoma (T/C 144%, 37.5 mg/kg).

Examination of Table III shows that introduction of a hydroxyl group enhances the cytotoxicity (**5** > **1**, **4** > **6**) while a bromine substituent diminishes it (**1** > **2**). Effects on antiviral activity are less consistent, but topsentin itself seems to represent an optimum to this point. The dihydroimidazole **3** shows cytotoxicity comparable to topsentin but only marginal antiviral activity.

Table III. Biological Activities of Topsentins

compd	P388[a] IC_{50}, μg/mL[b]	P388[a] T/C (mg/kg)[c]	HSV-1 (μg/disk)[d,e]	A-59 (μg/disk)[e,f]
1	2.0	132 (75)	0++ (200)	0+++ (20)
			0+ (50)	0+++ (2)
			0– (20)	0– (0.2)
2	7.0	126 (75)	0++ (200)	0++ (10)
			0– (50)	0– (5)
3	4.0			0+++ (20)
				0– (2)
4	4.0			0+++ (20)
				0– (2)
5[g]	0.3		0– (20)	0++ (20)
				0– (2)
6	12.0			0+++ (20)
				0++ (2)
				0– (0.2)
7	2.5		0– (20)	0– (20)
8	1.8		0– (20)	0– (20)
9	>20		0– (20)	0– (20)

[a] Murine leukemia cell line. [b] Concentration for 50% inhibition in vitro. [c] Test/control, survival in vivo. [d] Herpes simplex virus, type 1. [e] No cytotoxicity, 0; inhibitory action in plaque reduction assay: +, <25%; ++, 25–50%; +++, 50–75%; ++++, >75%. [f] Corona virus; mouse hepatitis virus (MHV), strain A-59. [g] Compound **5** was retested at the time compounds **7**–**9** were tested: P388, IC_{50} 16 μg/mL; A-59, 0– at 20 μg/disk.

The neotopsentin analogues (Table III) showed cytotoxicity generally comparable to that in the topsentin series but reduced antiviral activity. Neohydroxytopsentin was, however, far less cytotoxic.

Experimental Section

General Procedures. Melting points were measured by using a Thomas-Hoover capillary melting point apparatus and optical rotations by using a JASCO DIP-360 digital polarimeter. UV spectra were recorded on a Perkin-Elmer Lambda 3 UV/vis instrument and IR spectra on IBM IR/30 FTIR and Perkin-Elmer 1310 spectrophotometers. ^{1}H and ^{13}C NMR spectra were obtained on Varian XL-200, General Electric QE-300, Nicolet NT-360, and Bruker AM-360 NMR spectrometers; all chemical shifts are reported relative to Me_2SO (δ 2.49 for ^{1}H, 39.5 for ^{13}C). Low- and high-resolution electron ionization mass spectrometry (EIMS, 70 eV) were performed by using VG 70SE, Finnigan MAT 731, and CH5 mass spectrometers.

TLC was carried out on Whatman MKC-18F RP plates (200 μm) with 20% aqueous MeOH.

Sponge Collection. Sponge samples (all genus *Spongosorites*, Topsent 1896)[3] were collected by using the Johnson-Sea-Link I submersible and the *R/V Sea Diver* of the HBOI. Sponge samples 4-XII-84-1-23 (*Spongosorites* sp. 3) and 4-XII-84-1-22 (*Spongosorites* sp. 1) were obtained at −174 m at Chub Cay, Bahamas; sponge specimens 5-XII-84-3-4 (*Spongosorites ruetzleri*, Van Soest and Stentoft, 1988) and 23-VIII-85-1-39 (*Spongosorites* sp. 3) were collected at Goulding's Cay, Bahamas, at −355 and −229 m, respectively. The samples were immediately frozen and maintained below −20 °C until extraction.

Isolation of Topsentin (1) and Bromotopsentin (2). A. Sponge sample 4-XII-84-1-23 (243 g) was homogenized in methanol (500 mL, 2×). After filtration and evaporation at reduced pressure below 40 °C, a yellowish-brown extract (10.9 g, 5% from frozen sponge) was obtained. The extract (and that from 4-XII-84-1-22; cf. below) inhibited the growth of P388 mouse leukemia cells at 50 μg/mL and HSV-1 virus (plaque-reduction assay) at 200 μg/disk. A portion of the crude extract from 4-XII-84-1-23 (740 mg) was fractionated first by column chromatography (SiO_2, 75 g; $CHCl_3$–MeOH, 5:1). The biologically active, brown fraction (258 mg) gave yellow spots on TLC and was precipitated from chloroform–methanol as a bright-yellow mixture of topsentins (158 mg). Separation by RP-HPLC [Alltech C18, 10 μm, 10 × 250 mm, MeOH–H_2O, 3:1, 2.0 mL/min, UV (254 nm)] gave topsentin (**1**, 9.3 mg, 1.3% from the extract) and bromotopsentin (**2**, 139.8 mg, 18.9% from the extract).

B. From sponge sample 4-XII-84-1-22 (89.7 g) a yellowish-brown extract (3.20 g, 4% from frozen sponge) was obtained in the same manner, and from it topsentin (**1**, 11.5 mg, 0.4% from the extract) and bromotopsentin (**2**, 154 mg, 4.8% from the extract) were isolated in the same manner, except that Sephadex LH-20 ($CHCl_3$–MeOH, 3:2) was used to remove inactive impurities.

C. Sponge sample 5-XII-84-3-4 (264 g) was extracted twice with methanol–toluene (3:1), and the concentrated extract (11.3 g) was partitioned between pentane and 10% aqueous methanol. The alcohol layer was diluted to 30% water and extracted with methylene chloride. The aqueous layer was concentrated and partitioned between 1-butanol and water. A portion (200 mg) of the butanol-soluble fraction was chromatographed over RP material (Amicon silica C-8, 20–45 μm; 20% H_2O in MeOH) and monitored by antiviral bioassay (HSV-1). The antiviral fraction (123 mg) was separated further by RP-HPLC (C18, 5 μm; 20% H_2O in MeOH) into pure topsentin (**1**, 20 mg, 0.1% of wet weight) and bromotopsentin (**2**, 67 mg, 0.3%).

Topsentin (1): sparingly soluble, amorphous, bright-yellow solid, mp >250 °C; R_f 0.75 (TLC); IR (KBr) 3397 (OH, NH), 1626 (conj ketone), 1576, 1522, 1159, 1091 cm^{-1}; UV (95% EtOH) λ max nm (ε) 378 (17 300), 280 (13 500), 240 (sh, 19 200), 220 (sh, 31 500), 202 (41 000), changing upon addition of KOH to λ max nm (ε) 375 (3100), 300 (3400), 246 (5100), 208 (1800); ^1H and ^{13}C NMR, see Table I; HREIMS, *m/z* (relative intensity, LREIMS) 342.1107 (M, Δ 1.0 mmu for $C_{20}H_{14}N_4O_2$; 100), 209.0589 (Δ 0.0 mmu for $C_{12}H_7N_3O$, M − hydroxyindolyl − H; 37), 183.0794 (Δ 0.2 mmu for $C_{11}H_9N_3$; 10), 160.0395 (Δ 0.3 mmu for $C_9H_6NO_2$; 12), 133.0535 (Δ −0.7 mmu for C_8H_7NO; 48).

Bromotopsentin (2): sparingly soluble, bright-yellow crystals, mp 296–297 °C (from $CHCl_3$–MeOH, 9:1); R_f 0.58 (TLC); IR (KBr) 3403 (OH, NH), 1628 (ArC=O), 1585, 1522, 1156, 1105 cm^{-1}; UV (95% EtOH) λ max nm (ε) 378 (17 200), 286 (15 300), 254 (sh, 22 300), 237 (28 800), 208 (40 000), changing upon addition of KOH to λ max nm (ε) 375 (3500), 300 (4200), 234 (sh, 9700), 209 (19 000); ^1H and ^{13}C NMR, see Table I; HREIMS, *m/z* (relative intensity, LREIMS) 420.0214 (M, Δ 0.8 mmu for $C_{20}H_{13}{}^{79}BrN_4O_2$; 49), 342 (M − Br + H; 10), 286.9688 (Δ −0.6 mmu for $C_{12}H_6{}^{79}BrN_3O$, M − hydroxyindolyl − H; 10), 260.9894 (Δ −0.7 mmu for $C_{11}H_8{}^{79}BrN_3$, M − hydroxyindolylcarbonyl + H; 15), 160.0392 (Δ −0.6 mmu for $C_9H_6NO_2$; 47), 133.0526 (Δ −0.1 mmu for C_8H_7NO; 100), 105.0335 (Δ −0.5 mmu for C_7H_5O; 19).

Conversion of Bromotopsentin (2) to Topsentin (1). Bromotopsentin (**2**, 23.4 mg, 0.056 mmol) in absolute ethanol (2.0 mL) was stirred vigorously with 10% palladium on activated carbon (35 mg) under hydrogen at room temperature for 4 h. The reaction mixture was filtered, and washings were evaporated in vacuo to give a quantitative yield of topsentin (**1**, 19 mg, 0.056 mmol), identical with natural topsentin in LREIMS and ^1H NMR spectra.

Isolation of 4,5-Dihydro-6″-deoxybromotopsentin (3). Frozen sponge sample 23-VIII-85-1-39 (342 g) was homogenized and steeped repeatedly in methanol and 10% toluene followed by methanol. The alcohol layer was concentrated and repartitioned between 1-butanol and water, and the butanol-soluble fraction (1.42 g) was vacuum chromatographed over RP material (Amicon, silica gel C18, 20–45 μm) with 20% aqueous methanol. The yellow fraction was then subjected twice to RP-HPLC (C18, 5 μm, 20% H_2O in MeOH) to give **2** (434 mg, 0.12%) and **3** (20 mg, 0.005%): yellow powder; $[\alpha]^{24}D$ 198 ° (*c* 2.0, MeOH); UV (MeOH) λ max nm (ε) 328 (5700), 274 (8800), 214 (34 000), 198 (29 500); IR (KBr) 3620, 3390, 3280, 2920, 2860, 1665, 1570, 1450, 1420, 1332, 1240, 1160, 1120, 1100, 1020, 950, 805, and 750 cm^{-1}; ^1H and ^{13}C NMR, see Table I; LREIMS, *m/z* (relative intensity) 406 (95), 404 (100), 378 (41), 376 (39), 326 (10), 298 (6), 297 (7), 291 (10), 289 (9), 235 (6), 233 (6), 210 (12), 208 (10), 197 (10), 195 (10), 189 (5), 156 (12), 155 (19), 144 (28), 130 (14). Anal. Calcd for $C_{20}H_{13}{}^{79}BrN_4O$: M_r 404.0272 (M − 2H). Found: 404.0300 (HREIMS).

Preparation of 3-(Hydroxyacetyl)indole (12). 3-(Chloroacetyl)indole (**10**, 51 mg, 0.264 mmol), prepared in 34% yield according to a known procedure[19] and characterized by spectral data, was added to formamide–water (10:1, 5.0 mL) and stirred at 110 °C for 3.5 h. The reaction mixture was treated with a large excess of 14% aqueous ammonia and extracted with chloroform. After evaporation, the crude product (65 mg) was purified by RP medium-pressure (MP) LC (Waters C18; MeOH–H_2O, 3:1) to give **12** (45 mg, 0.257 mmol, 97% yield): colorless needles, mp 173–174 °C (EtOAc); ^1H NMR (200 MHz, 1% TFA in Me_2SO-d_6) δ 4.59 (s, 2 H), 7.20 (m, 2 H), 7.48 (m, 1 H), 8.18 (m, 1 H), 8.35 (d, 1 H, *J* = 3 Hz), 11.98 (br s, 1 H); LREIMS, *m/z* (relative intensity) 175 (26), 144 (100), 116 (10). Anal. Calcd for $C_{10}H_9NO_2$: M_r 175.0633. Found: 175.0633 (HREIMS).

Preparation of 3-(Chloroacetyl)-6-(benzyloxy)indole (11). 6-(Benzyloxy)indole (Sigma, 485 mg, 2.17 mmol) in dioxane (3.5 mL) containing pyridine (300 μL, 3.71 mmol) was stirred at 60 °C under nitrogen while chloroacetyl chloride (300 μL, 3.71 mmol) in dioxane (0.5 mL) was added dropwise during 1 h. The reaction mixture was then stirred for another 0.5 h and poured into diethyl ether (2 mL)–water (8 mL). The precipitate was collected by filtration and washed thoroughly with cold diethyl ether to yield **11** (303 mg, 1.01 mmol, 47%): orange solid; ^1H NMR (200 MHz, 1% TFA in Me_2SO-d_6) δ 4.83 (s, 2 H), 5.13 (s, 2 H), 6.94 (dd, 1 H, *J* = 1.6, 8.6 Hz), 7.04 (d, 1 H, *J* = 1.6 Hz), 7.3-7.5 (m, 5 H), 8.01 (d, 1 H, *J* = 8.6 Hz), 8.31 (d, 1 H, *J* = 2.8 Hz), 11.94 (br s, 1 H); LREIMS, *m/z* (relative intensity) 301 (2), 299 (8), 265 (1.3), 250 (1.3), 223 (2.4), 210 (2), 208 (5), 174 (1.8), 159 (6), 131 (7), 91 (100). Anal. Calcd for $C_{17}H_{14}{}^{35}ClNO_2$: M_r 299.0713. Found: 299.0713 (HREIMS).

Preparation of 3-(Hydroxyacetyl)-6-(benzyloxy)indole (13). A solution of **11** (344 mg, 1.15 mmol) in dioxane (10 mL) was added to formamide–water (10:1, 35 mL). The mixture was stirred at 110 °C for 10 h and then was worked up and purified as described for **12** above to give **13** (263 mg, 0.94 mmol, 82% yield): colorless prisms, mp 194–195 °C (EtOAc); ^1H NMR (200 MHz, 1% TFA in Me_2SO-d_6) δ 4.54 (s, 2 H), 5.13 (s, 2 H), 6.92 (dd, 1 H, *J* = 1.6, 8.6 Hz), 7.03 (d, 1 H, *J* = 1.6 Hz), 7.2–7.5 (m, 5 H), 8.02 (d, 1 H, *J* = 8.6 Hz), 8.21 (d, 1 H, *J* = 2.2 Hz), 11.77 (br s, 1 H); LREIMS, *m/z* (relative intensity) 281 (6.1), 250 (7.7), 190 (5.3), 159 (6.8), 131 (8.8), 91 (100). **Anal.** Calcd for $C_{17}H_{15}NO_3$: M_r 281.1052. Found: 281.1047 (HREIMS).

Syntheses of *O*-Benzyltopsentin (16), *O*-Benzylisotopsentin (17), *O,O′*-Dibenzylhydroxytopsentin (18), and Deoxytopsentin (6). A. Directly from (Hydroxyacetyl)indoles. Copper(II) acetate monohydrate (506 mg, 2.53 mmol) in 30% aqueous ammonia (10 mL) was added dropwise to a refluxing, stirred mixture of **12** (136 mg, 0.78 mmol) and **13** (56 mg, 0.20 mmol) in ethanol (20 mL) during 5 min. After addition was completed, the reaction mixture refluxed for another 10 min and then was allowed to cool to room temperature. Hydrogen sulfide gas was bubbled through the solution for 5 min. Filtration and evaporation gave a brown solid (185 mg). Column chromatography (SiO_2, 20 g; $CHCl_3$–MeOH, 50:1) followed by RP-MPLC (Waters

C18, 50 g; MeOH–H_2O, 3:1 → 4:1) and HPLC (Alltech C18, MeOH–H_2O, 7:3) gave **16** (7.9 mg, 0.018 mmol), **17** (5.9 mg, 0.014 mmol), **18** (1.6 mg, 0.003 mmol), and **6** (22.0 mg, 0.068 mmol) with recovered **12** (59.0 mg, 0.34 mmol) and **13** (22.8 mg, 0.081 mmol).

B. From Isolated Glyoxalyl Intermediates. Copper(II) acetate monohydrate (125 mg, 0.63 mmol) in 50% aqueous acetic acid (2.0 mL) was added to **12** (55 mg, 0.31 mmol) in ethanol (3.0 mL). The mixture was refluxed with stirring for 4 h and then was allowed to cool to room temperature, filtered through Celite, and evaporated at reduced pressure. Water was added, and the aqueous layer was extracted with ethyl acetate. The combined organic phase was washed with water, saturated aqueous sodium bicarbonate, and brine and then evaporated in vacuo to give nearly pure 3-glyoxalylindole (**14**, 54 mg). Similarly, **13** (55 mg, 0.20 mmol) in ethanol (2.0 mL) was treated with copper(II) acetate monohydrate (83 mg, 0.42 mmol) in 50% aqueous acetic acid. Workup gave nearly pure 3-glyoxalyl-6-(benzyloxy)indole (**15**, 58 mg).

3-Glyoxalylindole (**14**, 127 mg), prepared as above from **12** (107 mg, 0.61 mmol), and 3-glyoxalyl-6-(benzyloxy)indole (**15**, 58.7 mg), prepared as above from **13** (55 mg, 0.20 mmol), were dissolved in 75% aqueous ethanol (35 mL). Ammonia gas was bubbled through the solution for 15 min at room temperature and then for another 15 min under reflux. After cooling, the solvent was removed in vacuo, and the residue (162 mg) was purified by column chromatography (SiO_2, 20 g; $CHCl_3$–MeOH, 50:1) followed by RP-MPLC (Waters C18, 50 g; MeOH–H_2O, 1:1 → 3:1) and HPLC (Merck LiChrosorb NH_2, 7 μm; $CHCl_3$–MeOH, 10:1) to obtain **16** (10.3 mg, 0.019 mmol), **17** (8.4 mg, 0.016 mmol), **18** (5.0 mg, 0.009 mmol), and **6** (25.3 mg, 0.078 mmol).

***O*-Benzyltopsentin (16)**: bright-yellow solid; ^1H NMR, see Table I; LREIMS, m/z (relative intensity) 432 (13), 341 (16), 250 (23), 223 (17), 132 (24), 91 (100). Anal. Calcd for $C_{27}H_{20}N_4O_2$: M_r 432.1586. Found: 432.1594 (HREIMS).

***O*-Benzylisotopsentin (17)**: bright-yellow solid; ^1H NMR, see Table I; LREIMS, m/z (relative intensity) 432 (13), 341 (15), 315 (54), 144 (100), 117 (20), 91 (93). Anal. Found for $C_{27}H_{20}N_4O_2$: M_r 432.1594 (HREIMS).

***O*,*O*′-Dibenzylhydroxytopsentin (18)**: bright-yellow solid; ^1H NMR, see Table I; LREIMS, m/z (relative intensity) 538 (41), 447 (36), 315 (5), 289 (22), 250 (4), 223 (25), 132 (38), 117 (32), 91 (100). Anal. Calcd for $C_{34}H_{26}N_4O_3$: M_r 538.2005. Found: 538.2003 (HREIMS).

Deoxytopsentin (6): bright-yellow solid, mp >250 °C; UV (95% EtOH) nm (ε) 375 (20400), 273 (sh, 17700), 265 (sh, 18400), 252 (21700), 226 (sh, 31400), 209 (44000); ^1H NMR, see Table I; HREIMS, m/z (relative intensity, LREIMS) 326.1165 (0.2 mmu for $C_{20}H_{14}N_4O$; 10), 144 (43), 117.0572 (0.7 mmu for C_8H_7N; 15).

Conversion of *O*-Benzyltopsentin (16) to Topsentin (1). A solution of **16** (4.8 mg, 0.011 mmol) in absolute ethanol (10 mL) was stirred vigorously with 10% palladium on activated carbon (23 mg) under hydrogen at room temperature for 10 h. The reaction mixture was filtered through Celite and washed thoroughly with ethanol. After evaporation of ethanol, topsentin (**1**, 3.8 mg, 0.011 mmol) was obtained in quantitative yield. The synthesized topsentin was identical with natural topsentin in UV, ^1H NMR, and LREIMS spectra and in biological activities (P388, A-59 in vitro).

Conversion of *O*-Benzylisotopsentin (17) to Isotopsentin (4). A solution of **17** (2.5 mg, 0.006 mmol) in absolute ethanol (5 mL) was treated with 10% palladium on activated carbon (10 mg) as for **16** above to give **4** (2.0 mg, 0.006 mmol) in quantitative yield: yellow, amorphous solid; ^1H NMR, see Table I.

Synthesis of Hydroxytopsentin (5) from 3-(Hydroxyacetyl)-6-(benzyloxy)indole (13). Ammonia gas was bubbled for 15 min through a solution of **15** [848 mg, obtained as above from **13** (853 mg, 3.04 mmol)] in 75% aqueous ethanol (130 mL), and then the mixture refluxed for another 15 min. The precipitate was collected by filtration and washed with methanol to yield **18** (392 mg, 0.73 mmol). The combined filtrate and washings (553 mg after evaporation of the solvent) were purified by column chromatography (SiO_2, 100 g; $CHCl_3$–MeOH, 100:1) to give an additional 125 mg (0.23 mmol) of **18** (total yield 63% from **13**).

The precipitate of **18** (392 mg, 0.74 mmol) was dissolved in methanol (400 mL), and the solution was stirred vigorously with 10% palladium on activated carbon (600 mg) under hydrogen at room temperature for 2 h. Workup as described above for the syntheses of **1** and **4**, followed by purification (RP-MPLC, Waters C18; MeOH–H_2O; 1:1 → 4:1), gave **5** (233 mg, 0.65 mmol, 89%): yellow solid; ^1H NMR, see Table I; LREIMS, m/z (relative intensity) 358 (7), 225 (6), 199 (10), 160 (100), 133 (55). Anal. Calcd for $C_{20}H_{14}N_4O_3$: M_r 358.1066. Found: 358.1074 (HREIMS).

Preparation of 3-(Chloroacetyl)-5-(benzyloxy)indole (19). 5-(Benzyloxy)indole (Sigma, 3094 mg, 13.9 mmol) in dioxane (22 mL) containing pyridine (1.91 mL, 23.6 mmol) was stirred for 1 h at 65 °C under nitrogen, while chloroacetyl chloride (1.88 mL, 23.6 mmol) in dioxane (3.0 mL) was added dropwise. The reaction mixture was stirred for another 1 h, and then poured into diethyl ether (12.5 mL)–water (50 mL). The precipitate was collected by filtration and washed thoroughly with cold diethyl ether to yield **19** (2898 mg, 9.7 mmol, 70%): orange solid; ^1H NMR (200 MHz, 1% TFA–Me_2SO-d_6) δ 4.84 (s, 2 H), 5.12 (s, 2 H), 6.96 (dd, 1 H, J = 2.1, 8.8 Hz), 7.2–7.6 (m, 6 H), 7.78 (d, 1 H, J = 2.1 Hz), 8.38 (d, 1 H, J = 3.4 Hz), 12.10 (br s, 1 H); LREIMS, m/z (relative intensity) 301 (11), 299 (33), 264 (1), 223 (3), 91 (100). Anal. Found for $C_{17}H_{14}{}^{35}ClNO_2$: M_r 299.0715 (HREIMS).

Preparation of 3-(Hydroxyacetyl)-5-(benzyloxy)indole (20). A solution of **19** (2086 mg, 6.95 mmol) in dioxane (60 mL) was added to formamide–water (10:1, 215 mL), and the mixture was stirred at 110 °C for 6.5 h and then was worked up as for **12** above. Purification by column chromatography (SiO_2, 400 g; $CHCl_3$–MeOH, 50:1) gave **20** (1070 mg, 3.81 mmol, 55%): ^1H NMR (200 MHz, 1% TFA–Me_2SO-d_6) δ 4.57 (s, 2 H), 5.12 (s, 2 H), 6.95 (dd, 1 H, J = 2.0, 8.8 Hz), 7.2–7.6 (m, 6 H), 7.80 (d, 1 H, J = 2.0 Hz), 8.30 (d, 1 H, J = 2.6 Hz), 11.89 (br s, 1 H); LREIMS, m/z (relative intensity) 281 (71), 250 (71), 91 (100). Anal. Found for $C_{17}H_{15}NO_3$: M_r 281.1052 (HREIMS).

Synthesis of Neohydroxytopsentin (9). A solution of **20** (55 mg, 0.20 mmol) in ethanol (2.0 mL) was treated with copper(II) acetate monohydrate (83 mg, 0.42 mmol) in 50% aqueous acetic acid as described above for **15**. Workup gave nearly pure 3-glyoxalyl-5-(benzyloxy)indole (**21**, 56.2 mg).

Ammonia gas was bubbled for 15 min through a solution of **21** [194 mg, prepared from **20** (208 mg, 0.69 mmol)] in 75% aqueous ethanol (30 mL), and the mixture refluxed for another 15 min. After evaporation, the crude product was purified by column chromatography (SiO_2, 100 g; $CHCl_3$–MeOH, 100:1) to give *O*,*O*′-dibenzylneohydroxytopsentin (**24**, 66.3 mg, 0.12 mmol, 60%): yellow, amorphous solid; ^1H NMR (200 MHz, 1% TFA–Me_2SO-d_6) δ 5.19 (s, 2 H), 5.24 (s, 2 H), 6.96 (dd, 1 H, J = 2.2, 8.6 Hz), 7.05 (dd, 1 H, J = 2.1, 8.8 Hz), 7.2–7.6 (m, 12 H), 7.59 (d, 1 H, J = 2.2 Hz), 7.93 (s, 1 H), 8.02 (d, 1 H, J = 2.1 Hz), 8.06 (d, 1 H, J = 2.7 Hz), 9.03 (d, 1 H, J = 2.9 Hz), 11.48 (d, 1 H, J = 1.3 Hz), 12.33 (d, 1 H, J = 2.6 Hz); LREIMS, m/z (relative intensity) 538 (55), 448 (100), 315 (6), 289 (25), 250 (3), 223 (35), 91 (51).

A solution of **24** (60 mg, 0.11 mmol) in methanol (60 mL) was stirred vigorously with 10% palladium on activated carbon (90 mg) under hydrogen at room temperature for 3 h. Workup in the usual manner gave 57 mg of crude product, and purification by HPLC (ALTEX semi-Prep C18; MeOH–H_2O, 65:35; 4.5 mL/min) gave **9** (38 mg, 0.11 mmol, 95%): bright-yellow, amorphous solid; ^1H NMR (300 MHz, 1% TFA–Me_2SO-d_6) δ 6.73 (dd, 1 H, J = 1.8, 8.4 Hz), 6.77 (dd, 1 H, J = 2.1, 8.7 Hz), 7.25 (d, 1 H, J = 1.8 Hz), 7.26 (d, 1 H, J = 8.4 Hz), 7.35 (d, 1 H, J = 8.7 Hz), 7.68 (d, 1 H, J = 2.1 Hz), 7.82 (s, 1 H), 7.94 (d, 1 H, J = 2.4 Hz), 8.66 (d, 1 H, J = 1.8 Hz), 11.34 (br s, 1 H), 12.26 (d, 1 H, J = 1.5 Hz); LREIMS, m/z (relative intensity) 358 (52), 225 (17), 199 (20), 160 (18), 133 (100). Anal. Found for $C_{20}H_{14}N_4O_3$: M_r 358.1031 (HREIMS).

Syntheses of Neotopsentin (7) and Neoisotopsentin (8). Ammonia gas was bubbled (15 min, room temperature) through a solution of **14** [105 mg, prepared from **12** (107 mg, 0.61 mmol)] and **21** [56.2 mg, prepared from **20** (55 mg, 0.20 mmol)] in 75% aqueous ethanol (35 mL) and then for another 15 min under reflux. After cooling, the solvent was removed in vacuo, and the residue (168 mg) was purified by column chromatography (SiO_2, 20 g; $CHCl_3$–MeOH, 50:1) followed by RP-MPLC (Waters C18, 50 g; MeOH–H_2O, 1:1 → 3:1), then by HPLC (Merck LiChrosorb NH_2, 7 μm; $CHCl_3$–MeOH, 10:1) to obtain **22** (9.0 mg, 0.021 mmol, 11%), **23** (7.3 mg, 0.017 mmol, 9%), **24** (3.2 mg, 0.006 mmol, 3%), and **6** (27.4 mg, 0.084 mmol, 28%).

22: yellow, amorphous solid; ^{1}H NMR (200 MHz, 1% TFA–Me_2SO-d_6) δ 5.20 (s, 2 H), 7.05 (dd, 1 H, J = 2.0, 8.8 Hz), 7.21 (m, 2 H), 7.40 (d, 1 H, J = 8.8 Hz), 7.3-7.6 (m, 6 H), 7.92 (d, 1 H, J = 2.0 Hz), 8.03 (s, 1 H), 8.03 (d, 1 H, J = 8.2 Hz), 8.10 (d, 1 H, J = 2.8 Hz), 8.82 (d, 1 H, J = 2.9 Hz), 11.63 (d, 1 H, J = 2.5 Hz), 12.40 (d, 1 H, J = 2.4 Hz).

23: yellow, amorphous solid; ^{1}H NMR (200 MHz, 1% TFA–Me_2SO-d_6) δ 5.21 (s, 2 H), 6.94 (dd, 1 H, J = 1.8, 8.8 Hz), 7.30 (d, 1 H, J = 8.8 Hz), 7.31 (m, 2 H), 7.3-7.6 (m, 5 H), 7.51 (d, 1 H, J = 1.8 Hz), 7.59 (m, 1 H), 8.03 (s, 1 H), 8.04 (d, 1 H, J = 3.5 Hz), 8.32 (m, 1 H), 8.88 (d, 1 H, J = 3.0 Hz), 11.49 (d, 1 H, J = 1.4 Hz), 12.46 (d, 1 H, J = 3.3 Hz).

Compound **22** (8.0 mg, 0.019 mmol) in methanol (8.0 mL) was stirred vigorously with 10% palladium on activated carbon (12 mg) under hydrogen at room temperature for 3 h. The reaction mixture was filtered through Celite and washed thoroughly with ethanol. Evaporation of ethanol gave **7** (5.7 mg, 0.017 mmol, 90%): bright-yellow, amorphous solid; ^{1}H NMR (300 MHz, 1% TFA–Me_2SO-d_6) δ 6.77 (dd, 1 H, J = 2.1, 8.7 Hz), 7.17 (m, 2 H), 7.35 (d, 1 H, J = 8.7 Hz), 7.47 (d, 1 H, J = 8.1 Hz), 7.70 (d, 1 H, J = 1.8 Hz), 7.97 (s, 1 H), 7.99 (d, 1 H, J = 7.8 Hz), 8.06 (d, 1 H, J = 2.1 Hz), 8.72 (br s, 1 H), 11.59 (br s, 1 H), 12.23 (d, 1 H, J = 1.2 Hz); LREIMS, m/z (relative intensity) 342 (100), 209 (49), 183 (12), 160 (8), 133 (37). Anal. Found for $C_{20}H_{14}N_4O_2$: M_r 342.1118 (HREIMS).

Compound **23** (5.7 mg, 0.013 mmol) in methanol (5.7 mL) was treated with 10% palladium on activated carbon (8.6 mg) under hydrogen for 3 h and worked up in the same manner as for **7** above to give **8** (3.9 mg, 0.011 mmol, 86%): bright-yellow, amorphous solid; ^{1}H NMR (300 MHz, 1% TFA–Me_2SO-d_6) δ 6.72 (dd, 1 H, J = 1.8, 8.4 Hz), 7.25 (d, 1 H, J = 1.8 Hz), 7.26 (d, 1 H, J = 8.4 Hz), 7.30 (m, 2 H), 7.56 (m, 1 H), 7.82 (s, 1 H), 7.95 (d, 1 H, J = 2.1 Hz), 8.29 (m, 1 H), 8.83 (d, 1 H, J = 1.8 Hz), 11.33 (br s, 1 H), 12.45 (d, 1 H, J = 1.8 Hz); LREIMS, m/z (relative intensity) 342 (100), 225 (56), 199 (13), 144 (16), 117 (53). Anal. Found for $C_{20}H_{14}N_4O_2$: M_r 342.1114 (HREIMS).

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Registry No. 1, 112515-43-2; **2**, 112515-44-3; **3**, 116747-40-1; **4**, 116725-88-3; **5**, 116725-89-4; **6**, 112515-42-1; **7**, 116725-90-7; **8**, 116725-91-8; **9**, 116747-41-2; **10**, 28755-03-5; **11**, 116725-92-9; **12**, 2400-51-3; **13**, 116725-93-0; **14**, 7269-72-9; **15**, 116725-94-1; **16**, 116725-95-2; **17**, 116725-96-3; **18**, 116725-97-4; **19**, 37800-46-7; **20**, 116725-98-5; **21**, 116725-99-6; **22**, 116726-00-2; **23**, 116726-01-3; **24**, 116726-02-4; $Cu(OAc)_2$, 142-71-2; 6-(benzyloxy)indole, 15903-94-3; chloroacetyl chloride, 79-04-9; 5-(benzyloxy)indole, 1215-59-4.

Efficient Preparation of Some Biologically Active Substances from Natural and Nonnatural Aromatic Compounds by *m*-Chloroperbenzoic Acid Oxidation

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Six naturally occurring aromatic terpenoids and six nonnatural aromatic compounds were oxidized by *m*-chloroperbenzoic acid in chloroform to give 1,2- and 1,4-quinones or hydroxylated products in which vitamin K_1, insecticidal, piscicidal, and antifungal compounds were included. The present method is advantageous for obtaining different types of natural or nonnatural aromatic products having biological activity from the starting aromatic compounds in a one-step reaction.

In previous papers,[1-6] we reported hydroxylation at nonactivated carbon atoms of mono-, sesqui- and triterpenoids by *m*-chloroperbenzoic acid (MCPBA). Workup of this reaction was very simple and various hydroxylated compounds were obtained in one step. We have applied this simple method to some natural and nonnatural aromatic compounds and obtained quinones, some of which possessed piscicidal, antifungal, and insecticidal activity. In this paper we report the structures of the oxidation products and their biological activity.

Results and Discussion

The aromatic substances dissolved in chloroform were oxidized by MCPBA at room temperature or under reflux with stirring. Each mixture, after filtration of excess MCPBA and *m*-chlorobenzoic acid, was chromatographed on silica gel to give oxidation products. Table I shows the starting materials, oxidation products, and reaction conditions. Known compounds (**2**,[7,8] **4**,[9,10] **6**,[11] **10**,[11] **12**,[11] **15**,[12-15] **16**,[12] **20**,[16] **29**,[17] and **31**[18]) had properties consonant with

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