Isolation and Structures of Schleicherastatins 1−7 and Schleicheols 1 and 2 from the Teak Forest Medicinal Tree *Schleichera oleosa*[1,†]

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Bioassay (P-388 lymphocytic leukemia cell line)-guided separation of an extract prepared from the bark and stem of the Sri Lankan tree *Schleichera oleosa* led to the isolation of seven cancer cell growth inhibitory hydroxylated sterols designated schleicherastatins 1−7 (**1**−**7**) and two related sterols, schleicheols 1 and 2 (**8**, **9**). The structure of schleicherastatin 1 (**1**) was completely elucidated by X-ray crystal structure determination. Based upon that defined structure, the remaining new sterol structures were deduced by highfield (300 and 500 MHz) NMR and MS interpretations. In this new series of sterols, hydroxylation at C-22 appears to be important for promoting cancer cell growth inhibition.

The plant family Sapindaceae comprises some 2000 tropical and subtropical species that are primarily erect trees and shrubs (five of the 150 genera are lianas). One of these trees, *Schleicheria oleosa* (Lour.) Oken is a well-known medicinal plant[2a] in the teak forest of east Java (Indonesia) and is also used in West Bengal India, as a commercial lac host for obtaining sticklac for production of seedlac/shellac.[2b] In parts of southern India, *S. oleosa* is a prominent bee plant for nectar[2c] and is used for production of Kosum cake,[2d] an animal feedstock. It is also a timber (Ceylon Oak) source. In 1977, a collection of the stem wood and bark of *S. oleosa* from Thailand was found to give an extract that provided a confirmed level of antineoplastic activity against the P-388 lymphocytic leukemia in the U.S. National Cancer Institute's research programs.[3]

The present investigation was initiated with a 1982 Thailand re-collection of *S. oleosa* and brought to conclusion with a larger Sri Lankan re-collection of this plant. Dichloromethane fractions prepared from the methanol and dichloromethane−methanol extracts of the stem wood and bark showed, respectively, ED_{50} values of 2 and 6 μg/mL against the P-388 cell line. By 1986, the first[4] and only prior chemical study of this plant revealed the presence of lupeol- and betulinic acid-type triterpene constituents known to have antineoplastic properties.[5a−e] However, those components were not detected by the P-388 cell line bioassay of the Sri Lankan *S. oleosa*, and separations were continued until the new cell-growth inhibitory sterols schleicherastatins 1−7 (**1**−**7**) were isolated. This was accomplished by successive partitioning of the dichloromethane fraction prepared from the original extracts between methanol−water (9:1→1:1→1:1) and hexane, dichloromethane, and ethyl acetate, followed by a series of bioassay-directed chromatographic separations employing Sephadex LH-20 and Si gel column chromatographic steps, and culminating in HPLC and recrystallization procedures. In the process, the new sterols **8** and **9** were isolated and designated as schleicheols 1 and 2.

Results

Structure elucidations were begun using schleicherastatin 1 (**1**), utilizing principally HREIMS and highfield (300 and 500 MHz) NMR spectral methods. The molecular formula ($C_{30}H_{52}O_3$) of sterol **1** was deduced from the HREIMS molecular ion peak. The IR spectrum of this sterol showed an absorption band at 3404 cm^{-1} characteristic of a hydroxyl group. A close inspection of the ^1H and ^{13}C NMR spectra of sterol **1** (Table 1) by DEPT and $^1H-^{13}C$ COSY experiments revealed the presence of six methyl groups, including one primary (C-29), three secondary (C-21, C-26, and C-27), and two tertiary (C-18 and C-19) groups; nine methylenes (C-1, C-2, C-4, C-11, C-12, C-15, C-16, C-23, and C-28); 10 sp^3-hybridized methines (C-3, C-7−C-9, C-14, C-17, C-20, C-22, C-24, and C-25), including one methoxy (C-7) and two hydroxymethines (C-3 and C-22); two quaternary sp^3-carbons (C-10 and C-13); and one trisubstituted double bond (C-5 and C-6). The $^1H-^1H$ COSY analysis (Table 1) for the functional groups thus established led to partial structures shown by bold-faced lines in Figure 1, which were supported by HMBC correlations (Table 1). The connection of these partial structures was determined by the HMBC correlations shown in Figure 1. Based on this evidence, the planar structure of schleicherastatin **1** was elucidated.

The stereochemistry of sterol **1** was established by a combination of observed coupling constants and NOESY data (Table 1). The NOEs from H-19 to H-2α and H-4β and from H-3 to H-1α, H-2α, and H-4α and the coupling constants from H-3 to H-2α and H-4α (J = 11.2 Hz) suggested that the A-ring exists in a chair conformation with the 3-hydroxy and 10-methyl groups in an axial arrangement. The observed NOEs from H-7 to H-9 and H-14, from H-8 to H-19 and H-18, and from H-18 to H-11α and H-16β, as well as a coupling constant between H-7 and H-8 (J = 8.2 Hz) implied at the B- and C-rings exist in a twist-chair and chair conformation, respectively, with H-7 arranged cis-axially to H-9 and H-14, and trans-axially to H-8, and the 13-methyl group arranged axially. The configuration of C-20 and C-22 (*S* and *R*, respectively) was

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† Dedicated to the memory of Dr. Kenneth D. Paull (1942−1998), an outstanding research chemist devoted to anticancer drug discovery and development.

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Table 1. ^{1}H and ^{13}C NMR Assignments for Schleicherastatin 1 (**1**) in $CDCl_3$[a]

position		δ ^{1}H[b]	^{1}H−^{1}H COSY	NOESY	δ ^{13}C[c]	HMBC (C)[d]
1α	1.045 m		1*β*, 2*β*, 2α	3	37.0 (s)	2, 3
β	1.84 m		1α, 2*β*			3, 5
2α	1.85 m		1α, 2*β*, 3, 4α	3	31.6 (s)	10
β	1.50 m		1α, 1*β*, 2α, 3	4*β*, 19		
3	3.54 tt	11.2 (2*β*, 4*β*), 5.0 (2α, 4α)	2α, 2*β*, 4α, 4*β*	1α, 2α, 4α, 3-OH	71.5 (t)	
4α	2.35 ddd	13.0 (4*β*), 5.0 (3), 2.0 (2α)	2α, 3, 4*β*	3, 6	42.0 (s)	2, 3, 5, 6
β	2.28 ddt	13.0 (4α), 11.2 (3), 2.0 (6, 7)	3, 4α, 6, 7	2*β*, 19		2, 3, 5, 6
5					144.0 (q)	
6	5.44 t	2.0 (4*β*, 7)	4*β*, 7	4α, 7, OMe	121.4 (t)	4, 8, 10
7	3.35 dt	8.2 (8), 2.0 (4*β*, 6)	4*β*, 6, 8	6, 9, 14, 15α OMe	81.9 (t)	5, 6, 8, 14, OMe
8	1.52 m		7	18, 19, OMe	37.2 (t)	7, 9
9	1.02 m		11*β*, 11α	7, 11α, 14	48.4 (t)	7, 10, 11, 19
10					36.5 (q)	
11α	1.54 m		9, 11*β*, 12α, 12*β*	9, 12*β*	21.2 (s)	9
β	1.461 m		9, 11α, 12α, 12*β*	12*β*, 18, 19		8, 12
12α	1.16 td	12.4 (12*β*, 11*β*), 4.0 (11α)	11*β*, 11α, 12*β*, 18	17	39.6 (s)	13, 17, 18
β	2.01 dt	12.4 (12α), 3.5 (11α, 11*β*)	11*β*, 11α, 12a	11*β*, 11α, 18, 21		
13					43.2 (q)	
14	1.12 m		15*β*, 15α	7, 9, 15α	56.0 (t)	7, 8, 9, 13, 15, 18
15α	1.69 m		14, 15*β*, 16*β*, 16α	7, 14, OMe	25.8 (s)	13, 14
β	1.465 m		14, 15*β*, 16*β*, 16α			14, 16
16α	1.74 m		15*β*, 15α, 16*β*, 17	17	27.7 (s)	13, 14, 15
β	1.37 m		15*β*, 15α, 16α, 17	18, 22		14, 17, 20
17	1.07 m		16*β*, 16α	12α, 16α, 21	52.5 (t)	21
18	0.71 s		12α	8, 11*β*, 12*β*, 16*β*, 20, 21	11.9 (p)	12, 13, 14, 17
19	1.041 s			2*β*, 4*β*, 8, 11*β*	19.0 (p)	5, 9, 10
20	1.70 dqd	10.8 (17), 6.9 (21), 3.5 (22)	21	18, 21, 22	42.5 (t)	13
21	0.92 d	6.9 (20)	20, 22	12*β*, 17, 18, 20, 22-OH	12.4 (p)	17, 20, 22
22	3.73 br ddd	10.8 (23*β*), 3.5 (20), 1.5 (23α)	20, 23α, 23*β*	16*β*, 20, 22-OH	71.3 (t)	
23A	1.037 m		22, 23*β*, 24		29.9 (s)	24
B	1.244 m		22, 23α			25
24	1.254 m		23α, 25, 28*β*		41.4 (t)	22, 28
25	1.79 heptet d	6.9 (26, 27), 3.5 (24)	24, 26, 27		28.8 (t)	
26	0.80 d	6.9 (25)	25		17.6 (p)	24, 25, 27
27	0.894 d	6.9 (25)	25		20.5 (p)	24, 25, 26
28A	1.248 m		29		23.5 (s)	23, 24, 29
B	1.39 m		24, 29			23, 24, 29
29	0.89 t	7.2 (28α, 28*β*)	28α, 28*β*		11.8 (p)	24, 28
OMe	3.28 s			6, 7, 8, 15α		7
3-OH	1.60 br s			3		
22-OH	1.60 br s			21, 22		

[a] Measured at 500 and 127.5 MHz for ^{1}H and ^{13}C, respectively. [b] ^{1}H Chemical-shift values (δ ppm from TMS) followed by multiplicity and then the coupling constant (*J*/Hz), assigned by ^{1}H− ^{13}C COSY and homonuclear *J*-resolved 2D spectroscopy. Figures in parentheses indicate a proton coupling with that position. [c] Letters, p, s, t, and q, in parentheses indicate respectively primary, secondary, tertiary, and quaternary carbons assigned by DEPT. [d] Long-range ^{1}H−^{13}C correlation from H to C observed in the HMBC.

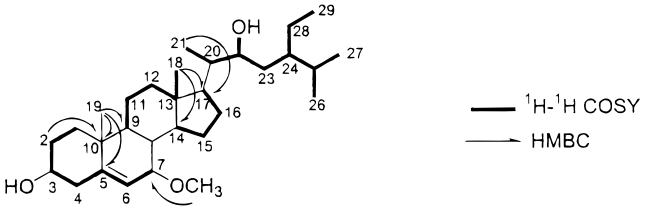


Figure 1. Selected ^{1}H−^{1}H COSY and HMBC correlations found for schleicherastatin 1 (**1**).

suggested by the NOEs from H-21 to H-12α, H-17, H-18, and OH-22; from H-20 to H-18; and from H-22 to H-16α and H-20. The utility of the ^{13}C NMR chemical-shift difference of C-20 has been reported to differentiate (22*R*)- and (22*S*)-hydroxycholesterols, where the observed chemical shifts of C-20 were δ 42.6 and 40.3, respectively.[6] Application of this chemical shift rule for sterol **1** (δ 42.51 for C-20) supported the configuration of C-22 described above.

To determine the stereochemistry of the 24-position, an X-ray crystal structure determination was performed with a single crystal of sterol **1** (obtained by recrystallization from methanol). Results of the X-ray structure elucidation (Figure 2) allowed definitive assignment of all asymmetric centers, ring conformations, and the overall structure of

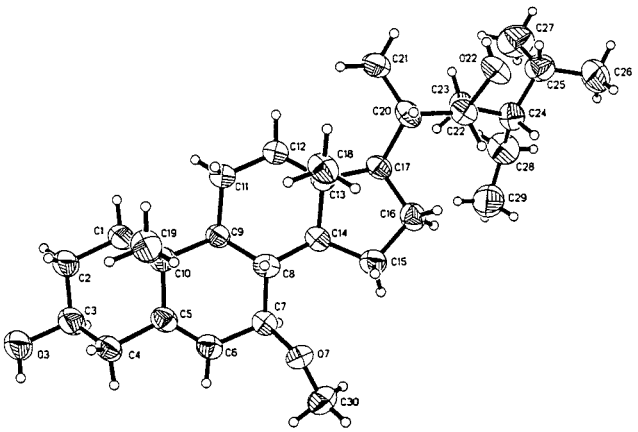


Figure 2. Perspective structure of schleicherastatin 1 (**1**) as a 40% thermal ellipsoid probability plot.

schleicherastatin 1 (**1**). The presence of the $\Delta^{5,6}$ double bond was confirmed by the shortened bond distances between these atoms (1.321 Å). Using the known absolute configuration of the steroid nucleus as a reference, the configuration at C-20 and C-22 as *S* and *R*, respectively, was readily corroborated. Similarly, the assignment of *R* to the absolute configuration at C-24 was established. Thus,

Table 2. The ^{1}H NMR Spectral Results for Schleicherastatins 2−5 (**2**−**5**) in $CDCl_3$[a,b]

position	2		3		4		5	
3	3.61 tt	11.0 (2β, 4β), 5.0 (2α, 4α)	3.55 tt	11.0 (2β, 4β), 5.0 (2α, 4α)	3.55 tt	11.0 (2β, 4β), 5.0 (2α, 4α)	3.68 tt	11.0 (2β, 4β), 5.0 (2α, 4α)
4α	2.36 ddd	13.0 (4β), 5.0 (3), 1.8 (2α)	2.36 ddd	13.0 (4β), 5.0 (3), 1.8 (2α)	2.36 ddd	13.0 (4β), 5.0 (3), 1.8 (2α)	2.51 ddd	13.0 (4β), 5.0 (3), 1.8 (2α)
β	2.30 ddt	13.0 (4α), 11.0 (3), 1.8 (6, 7)	2.28 ddt	13.0 (4α), 11.0 (3), 1.8 (6,7)	2.28 ddt	13.0 (4α), 11.0 (3), 1.8 (6,7)	2.40 ddt	13.0 (4α), 11.0 (3), 1.8 (6,7)
6	5.74 dd	4.8 (7), 1.8 (4β)	5.44 t	1.8 (4β, 7)	5.44 t	1.8 (4β, 7)	5.70 d	1.8 (4β)
7	3.30 br t	4.8 (6, 8)	3.36 dt	8.2 (8), 1.8 (4β, 6)	3.36 dt	8.2 (8), 1.8 (4β, 6)		
8							2.25 t	11.0 (9, 14)
12β	1.97 dt	12.7 (12α), 4.0 (11α, 11β)	2.01 dt	12.7 (12α), 4.0 (11α, 11β)	2.01 dt	12.7 (12α), 4.0 (11α, 11β)	2.04 dt	12.7 (12α), 4.0 (11α, 11β)
18	0.69 s		0.71 s		0.71 s		0.71 s	
19	0.99 s		1.04 s		1.04 s		1.21 s	
21	0.93 d	6.8 (20)	0.92 d	6.8 (20)	0.93 d	6.8 (20)	0.93 d	6.8 (20)
22	3.73 br d	10.8 (23β)	3.76 br d	10.8 (23β)	3.77 br d	10.8 (23β)	3.74 br d	10.8 (23β)
26	0.80 d	6.8 (25)	0.84 d	6.8 (25)	0.78 d	6.8 (25)	0.80 d	6.8 (25)
27	0.90 d	6.8 (25)	0.88 d	6.8 (25)	0.92 d	6.8 (25)	0.89 d	6.8 (25)
28			0.83 d	6.8 (24)	0.85 d	6.8 (24)		
29	0.89 t	6.8 (28)					0.88 t	7.0 (28)
OMe	3.36 s		3.28 s		3.28 s			

[a] Measured at 300 MHz. [b] As in Table 1.

Table 3. The ^{1}H NMR Spectral Assignments for Schleicherastatins 6 and 7 (**6** and **7**) and Schleicheols 1 and 2 (**8** and **9**)

position	6		7		8		9	
3	3.68 tt	11.0 (2β, 4β), 5.0 (2α, 4α)	3.68 tt	11.0 (2β, 4β), 5.0 (2α, 4α)	3.67 tt	11.0 (2β, 4β), 5.0 (2α, 4α)	3.64 tt	11.0 (2β, 4β), 5.0 (2α, 4α)
4α	2.52 ddd	13.0 (4β), 5.0 (3), 1.8 (2α)	2.52 ddd	13.0 (4β), 5.0 (3), 1.8 (2α)	2.35 ddd	13.0 (4β), 5.0 (3), 1.8 (2α)	2.36 ddd	13.0 (4β), 5.0 (3), 1.8 (2α)
4β	2.40 ddt	13.0 (4α), 11.0 (3), 1.8 (6, 7)	2.40 ddt	13.0 (4α), 11.0 (3), 1.8 (6, 7)	2.28 ddt	13.0 (4α), 11.0 (3), 1.8 (6, 7)	2.30 ddt	13.0 (4α), 11.0 (3), 1.8 (6, 7)
6	5.70 d	1.8 (4β)	5.70 d	1.8 (4β)	5.44 t	1.8 (4β, 7)	5.74 dd	4.8 (7), 1.8 (4β)
7					3.36 dt	8.2 (8), 1.8 (6, 4β)	3.29 br t	4.8 (6, 8)
8	2.25 t	11.0 (9, 14)	2.25 t	11.0 (9, 14)				
12β	2.04 dt	12.7 (12α), 4.0 (11α, 11β)	2.04 dt	12.7 (12α), 4.0 (11α, 11β)	2.01 dt	12.7 (12α), 3.8 (11α, 11β)	1.97 dt	12.7 (12α), 3.8 (11α, 11β)
18	0.71 s		0.71 s		0.68 s		0.66 s	
19	1.21 s		1.21 s		1.04 s		0.99 s	
21	0.93 d	6.8 (20)	0.93 br d	6.8 (20)	0.92 d	6.8 (20)	0.93 d	6.8 (20)
22	3.76 br d	10.8 (23β)		10.8 (23β)				
26	0.84 d	6.8 (25)	0.77 d	6.8 (25)	0.82 d	6.8 (25)	0.82 d	6.8 (25)
27	0.88 d	6.8 (25)	0.91 d	6.8 (25)	0.86 d	6.8 (25)	0.86 d	6.8 (25)
28	0.83 d	6.8 (24)	0.85 d	6.8 (24)				
29					0.85 t	6.8 (28)	0.85 t	6.8 (28)
OMe					3.28 (s)		3.36 s	

[a,b] As in Table 2.

schleicherastatin 1 (**1**) was determined to be 3β, 22α-dihydroxy-7β-methoxy-24β-ethyl-cholest-5-ene.

The structures of schleicherastatins 2−7 were deduced from the X-ray crystal structure of schleicherastatin 1 (**1**), combined with HREIMS and highfield NMR spectral interpretations. With schleicherastatin 2 (**2**), the NOEs from H-7 to H-8 and H-15β indicated this sterol to be a diastereoisomer of schleicherastatin 1 at C-7, that is, 24β-ethyl-7α-methoxycholest-5-ene-3β, 22α-diol. The coincidence of the ^{1}H and ^{13}C NMR chemical shifts of C-1−C-22 in the spectra of schleicherastatins 3 (**3**) and 4 (**4**) with those of sterol **1** implied that the stereochemistry at each asymmetric center, except for C-24, was the same in both compounds. Furthermore, the NMR evidence showed that the C-24 ethyl group of sterol **1** was replaced in sterol **3** and also in sterol **4** by a methyl group. The similarity of the chemical shifts for the carbon signals of schleicherastatins 3 and 4, except between C-24 and C-28, suggested that they had a diastereomeric relationship at C-24. The C-20 and C-24 carbon signals in cholestane systems show characteristic differences for 24*R*- and 24*S*-isomers, with those shifts in the 24*R* isomer being at slightly higher field than those of the 24*S* isomer.[7] Illustrative is the case of 24-methylcholesterols with ^{13}C NMR resonances for C-20 and C-24 at δ 35.9 and 38.9 in the 24*R* isomer compared to δ 36.1 and 39.1 in the 24*S* isomer. Furthermore, chemical shifts for the C-25 and C-26 signals (δ 32.4 and 18.2, respectively) in the C-24*R* sterol appear at lower field than in the C-24*S* epimer (δ 31.5 and 17.6, respectively). Thus, application of these guidelines to schleicherastatins 3 (**3**) and 4 (**4**) indicated assignment of *R* and *S*, respectively, to the C-24 positions. These assignments were also supported by the elution pattern in reversed-phase HPLC, where the C-24*S* epimer generally elutes prior to the C-24*R* epimer.[8] Schleicherastatins 3 and 4 were assigned as 3β, 22α-dihydroxy-7β-methoxy-24β-methyl- and -24α-methyl-cholest-5-ene, respectively.

Schleicherastatin 5 (**5**) was found to have the molecular formula $C_{29}H_{48}O_3$ (by HREIMS). The IR spectrum exhibited absorption bands for hydroxyl groups and a conjugated ketone. The ^{1}H and ^{13}C NMR spectra of sterol **5** (Tables 2 and 4) revealed that the methoxyl group in schleicherastatins 1 and 2 was replaced by a ketone in sterol **5**. The chemical shifts of all the carbon signals except for C-5−C-9 and C-19 and the coupling relationships of H-3 (tt, $J_{2\beta,3} = J_{3,4\beta} = 11.0$ Hz and $J_{2\alpha,3} = J_{3,4\alpha} = 5.0$ Hz) and H-22 (br d, $J_{22,23\beta} = 10.8$ Hz) in sterol **5** were compatible with those of schleicherastatins 1 and 2. In addition, the coupling constants ($J_{8,9} = J_{8,14} = 11.0$ Hz) from H-8 to H-9 and H-14 implied the configuration of all the asymmetric centers in

Table 4. ^{13}C NMR Chemical Shift Assignments for Schleicherastatins 2−7 (**2**−**7**) and Schleicheols 1 and 2 (**8** and **9**)

position	2	3	4	5	6	7	8	9
1	36.7 (s)	37.0 (s)	37.0 (s)	36.4 (s)	36.4 (s)	36.4 (s)	37.0 (s)	36.7 (s)
2	31.5 (s)	31.6 (s)	31.7 (s)	31.2 (s)	31.2 (s)	32.2 (s)	31.7 (s)	31.5 (s)
3	71.4 (t)	71.5 (t)	71.6 (t)	70.5 (t)	70.6 (t)	70.6 (t)	71.6 (t)	71.4 (t)
4	42.3 (s)	42.0 (s)	42.0 (s)	41.8 (s)	41.8 (s)	41.8 (s)	42.0 (s)	42.3 (s)
5	146.2 (q)	144.0 (q)	144.0 (q)	165.2 (q)	164.8 (q)	165.2 (q)	144.0 (q)	146.1 (q)
6	120.7 (t)	121.4 (t)	121.5 (t)	126.0 (t)	126.1 (t)	126.1 (t)	121.6 (t)	120.8 (t)
7	73.8 (t)	81.9 (t)	81.9 (t)	202.2 (q)	201.9 (q)	199.8 (q)	81.9 (t)	73.9 (t)
8	37.2 (t)	37.2 (t)	37.3 (t)	45.4 (t)	45.5 (t)	45.5 (t)	37.2 (t)	37.2 (t)
9	42.7 (t)	48.4 (t)	48.5 (t)	49.9 (t)	50.0 (t)	49.9 (t)	48.5 (t)	42.7 (t)
10	37.5 (q)	36.5 (q)	36.5 (q)	38.3 (q)	38.3 (q)	38.4 (q)	36.5 (q)	37.4 (q)
11	20.8 (s)	21.2 (s)	21.3 (s)	21.2 (s)	21.2 (s)	21.2 (s)	21.2 (s)	20.8 (s)
12	39.1 (s)	39.7 (s)	39.7 (s)	38.6 (s)	38.7 s)	38.7 (s)	39.7 (s)	39.0 (s)
13	42.4 (q)	43.2 (q)	43.3 (q)	43.4 (q)	43.4 (q)	43.4 (q)	42.9 (q)	42.1 (q)
14	48.8 (t)	56.0 (t)	56.1 (t)	49.5 (t)	49.5 (t)	49.5 (t)	56.5 (t)	49.1 (t)
15	24.4 (s)	25.8 (s)	25.9 (s)	26.4 (s)	26.4 (s)	26.4 (s)	25.7 (s)	24.3 (s)
16	27.5 (s)	27.6 (s)	27.9 (s)	27. 8 (s)	27.7 (s)	27.9 (s)	28.5 (s)	28.3 (s)
17	52.7 (t)	52.6 (t)	52.7 (t)	51.7 (t)	51.9 (t)	51.8 (t)	55.6 (t)	55.7 (t)
18	11.5 (p)	11.9 (p)	11.9 (p)	12.0 (p)	12.0 (p)	12.0 (p)	11.9 (p)	11.5 (p)
19	18.3 (p)	19.0 (p)	19.1 (p)	17.3 (p)	17.3 (p)	17.3 (p)	19.0 (p)	18.3 (p)
20	42.5 (t)	42.3 (t)	42.8 (t)	42.4 (t)	42.2 (t)	42.7 (t)	36.1 (t)	36.2 (t)
21	12.3 (p)	12.4 (p)	12.5 (p)	12.4 (p)	12.5 (p)	12.5 (p)	18.9 (p)	18.8 (p)
22	71.3 (t)	71.0 (t)	71.9 (t)	71.3 (t)	71.0 (t)	71.9 (t)	34.0 (s)	33.9 (s)
23	29.9 (s)	34.1 (s)	34.6 (s)	29.9 (s)	34.1 (s)	34.6 (s)	26.1 (s)	26.0 (s)
24	41.4 (t)	34.6 (t)	35.4 (t)	41.4 (t)	34.6 (t)	35.4 (t)	45.9 (t)	45.8 (t)
25	28.7 (t)	33.4 (t)	29.7 (t)	28.7 (t)	33.4 (t)	29.7 (t)	29.2 (t)	29.1 (t)
26	17.5 (p)	18.4 (p)	16.2 (p)	17.6 (p)	18.4 (p)	16.2 (p)	19.0 (p)	19.0 (p)
27	20.6 (p)	19.9 (p)	21.2 (p)	20.5 (p)	19.9 (p)	21.1 (p)	19.9 (p)	19.8 (p)
28	23.6 (s)	15.0 (p)	15.7 (p)	23.5 (s)	15.0 (p)	15.7 (p)	23.1 (s)	23.1 (s)
29	11.9 (p)			11.8 (p)			12.0 (p)	12.0 (p)
OMe	56.7 (p)	54.9 (p)	54.9 (p)				54.9 (p)	56.8 (p)

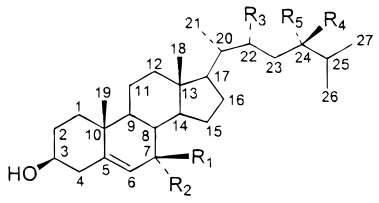
[a] Measured at 75.4 MHz. [b] As *c* in Table 1.

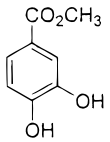
schleicherastatin 5 was the same as that of the asymmetric centers (except for C-7) in sterols **1** and **2**. This evidence led to stereostructure **5** for schleicherastatin 5, corresponding to 3*β*, 22α-dihydroxy-7-oxo-24*β*-ethyl-cholest-5-ene.

The molecular formula ($C_{28}H_{46}O_3$) derived by HREIMS was found to be the same for schleicherastatins 6 (**6**) and 7 (**7**). The ^1H and ^{13}C NMR spectra of both compounds closely resembled those of sterol **5** except for the chemical shifts of the side chain at C-23−C-28 (Tables 3 and 4) and revealed that the 24-ethyl group in sterol **5** was replaced by a methyl group in both **6** and **7**. Differences in the chemical shifts for carbon signals corresponding to C-24−C-28 in sterols **6** and **7** agreed with those of sterols **3** and **4**, respectively. This evidence allowed assignments of the C-24*R*- and C-24*S*-methyl substitution in schleicherastatins **6** and **7**. Schleicherastatin 6 was thus assigned 24*β*-methyl-3*β*,22α-dihydroxy-7-oxo-cholest-5-ene, while schleicherastatin 7 is 24α-methyl-3*β*,22α-dihydroxy-7-oxo-cholest-5-ene.

Schleicheols 1 (**8**) and 2 (**9**) both proved to have the molecular formula $C_{30}H_{52}O_2$, with their ^1H and ^{13}C NMR spectra closely resembling those of schleicherastatins 1 (**1**) and 2 (**2**), respectively. The exception was the chemical shifts of the side-chain carbons at C-20−C-24 and C-26 (Tables 3 and 4), which showed that the 22-hydroxymethine in sterols **1** and **2** was replaced by a methylene in schleicheols **8** and **9**. In addition, the chemical shifts of the side-chain carbons were found to be in accord with those of *β*-sitosterol.[9] On the basis of this evidence, the structures of schleicheols 1 (**8**) and 2 (**9**) were assigned as 24*β*-ethyl-7*β*-methoxy- and 3*β*-hydroxy-7α-methoxy-24*β*-ethyl-cholest-5-ene, respectively.

Because the ^{13}C NMR chemical shifts of sterol **10** were in accord with those of C-1−C-19 in sterol **5** and the side chain (C-20−C-29) in sterols **8** and **9**, it was considered to be 7-oxo-*β*-sitosterol. Although comparison of NMR spectral data for sterol **10** with published values[9] for 7-oxo-*β*-sitosterol showed a few differences in the chemical shifts of carbon signals for C-5, C-7, C-12, and C-13, detailed NMR spectral analysis confirmed compound **10** to be 7-oxo-*β*-sitosterol. The other known compound (**11**) was identified as the previously known methyl protocatechuate by direct comparison with an authentic sample.





11

1 $R_1 = OCH_3$, $R_2 = H$, $R_3 = OH$, $R_4 = CH_2CH_3$, $R_5 = H$

2 $R_1 = H$, $R_2 = OCH_3$, $R_3 = OH$, $R_4 = CH_2CH_3$, $R_5 = H$

3 $R_1 = OCH_3$, $R_2 = H$, $R_3 = OH$, $R_4 = CH_3$, $R_5 = H$

4 $R_1 = OCH_3$, $R_2 = H$, $R_3 = OH$, $R_4 = H$, $R_5 = CH_3$

5 $R_1 = R_2 = O$, $R_3 = OH$, $R_4 = CH_2CH_3$, $R_5 = H$

6 $R_1 = R_2 = O$, $R_3 = OH$, $R_4 = CH_3$, $R_5 = H$

7 $R_1 = R_2 = O$, $R_3 = OH$, $R_4 = H$, $R_5 = CH_3$

8 $R_1 = OCH_3$, $R_2 = H$, $R_3 = H$, $R_4 = CH_2CH_3$, $R_5 = H$

9 $R_1 = H$, $R_2 = OCH_3$, $R_3 = H$, $R_4 = CH_2CH_3$, $R_5 = H$

10 $R_1 = R_2 = O$, $R_3 = H$, $R_4 = CH_2CH_3$, $R_5 = H$

Discussion

The cancer cell growth inhibitory properties of sterols **1**−**10** and phenol **11** were examined using the murine P-388 lymphocytic leukemia cell line and a selection of human cancer cell lines. Schleicherastatins 1−7 (Table 5) and phenol **11** (P-388 ED_{50} 1.4 μg/mL) exhibited significant

Table 5. Cancer-Cell-Growth-Inhibitory Evaluation (GI_{50}) of the Schleicherastatins (**1**−**7**) and Schleicheols 1 and 2 (**8** and **9**)[a]

cancer cell line	schleicherastatin							schleicheol	
GI_{50} μg/mL	1(**1**)	2 (**2**)	3 (**3**)	4 (**4**)	5 (**5**)	6 (**6**)	7 (**7**)	1 (**8**)	2 (**9**)
leukemia P-388	0.19	0.72	1.2	1.2	0.34	0.78	0.78	14	15
CNS SF-295								3.2	1.9
colon KM 20L2								2.5	1.2
lung NCI−H460								1.8	2.2
ovary OVCAR-3								2.1	2.5
pancreas BXPC-3								3.3	1.4
prostate								1.8	1.6

[a] DMSO was used as vehicle.

inhibitory activity against the murine P-388 lymphocytic leukemia, and schleichols 1 and 2 (Table 5) showed marginal activity against a mini-panel of human tumor cell lines. Extension to the human cell lines and in vivo evaluations using the schleicherastatins will require their reisolation in sufficient quantities. Present results indicate that the 22-hydroxy group is important for activity in such sterols and that the sterols with the 24-ethyl side-chain substituent are more active than those with the 24-methyl group.

Early antineoplastic evaluations of β-sitosterol found that this simple sterol significantly inhibits the Walker carcinoscarcoma and Lewis lung cancer in vivo models.[10] Analogous evaluation of schleicherastatins 1 and 2 might also lead to in vivo antineoplastic activity. That prospect would also be interesting to explore with the other stigmastenes (**5, 8**−**10**) and ergostenes (**3**−**7**). From a broader perspective, many examples of steroids oxygenated at C-22 with cancer cell growth inhibitory properties are well-known to occur in certain plants[11] and marine invertebrates.[12]

Experimental Section

General Experimental Procedures. All solvents employed for chromatographic purposes were redistilled. Protocatechuic acid was obtained from Sigma−Aldrich Chemical Co. Sephadex LH-20 used for gel permeation and partition column chromatography was obtained from Pharmacia Fine Chemicals AB. Liquid chromatography over Si gel (mesh 230−400) was performed in a medium-pressure column. HPLC was conducted with a Waters ALC-200 instrument equipped with a differential refractometer (R401) and Shim-pack PREP−ODS (25 cm × 20 mm i.d.). Analtyical TLC was performed using precoated E. Merck aluminum sheets (DC−Alufolien Kieselgel 60 F_{254}, 0.2 mm) with the solvent systems dichloromethane−methanol 19:1 or 9:1. The plates were viewed under a UV lamp and developed by spraying with 10% sulfuric acid followed by heating. Optical rotations (all in chloroform solution) were obtained with a JASCO ORD/UV-5 spectropolarimeter. UV spectra were recorded on a Shimadzu spectrophotometer and IR spectra on a Perkin−Elmer 1720x FT−IR spectrometer 1720X. CD spectra were recorded with a JASCO J-500A spectrometer. NMR spectra were recorded at 27 °C with a Varian XL-300 or a Varian UNITY INOVA-500 spectrometer with tetramethylsilane (TMS) as an internal reference. EIMS was determined on a Hitachi M-4000H mass spectrometer. X-ray data were collected on an Enraf−Nonius CAD4 computer-controlled κ axis difractometer equipped with a graphite crystal, incident beam monochromator.

Plant Collection. The initial and the first re-collections of *Schleicheria oleosa* (Lour.) Oken (Sapindaceae) were conducted in Thailand in 1977 and 1982, respectively. The second re-collection of *S. oleosa*, amounting to 184 kg, was performed in Sri Lanka in May 1982, and that re-collection led to the isolation of schleicherastatins 1−7 (**1**−**7**) and schleicheols 1 and 2 (**8** and **9**), along with the 7-oxo-β-sitosterol (**10**) and methyl protocatechuate (**11**). The original and re-collections of *S. oleosa* were pursued as part of the joint U.S. National Cancer Institute/U.S. Department of Agriculture research program under the direction of Drs. John D. Douros, Matthew I. Suffness/Robert E. Purdue, and James A. Duke. The Sri Lankan re-collection was assisted by Dr. K. Balasubramaniam. A voucher specimen of *S. oleosa* has been deposited in the medicinal plant resources laboratory of the U.S. Department of Agriculture, Beltsville, MD.

Extraction and Initial Separation of *S. oleosa*. The 1982 re-collection of *S. oleosa* in Thailand amounting to 66.5 kg was extracted with 1:1 dichloromethane−methanol (104 L) for 7 days. After 7 days, (25% by volume) of water was added to the extract solution, and the phases were separated. The resulting, dried dichloromethane extract (196 g, P-388 ED_{50} 2 μg/mL) was partitioned between methanol−water (9:1; 2 L) and hexane (3 × 1 L) yielding 80 g of a P-388-inactive hexane fraction. The 9:1 methanol−water solution was diluted to 1:1 and extracted with methylene chloride (3 × 1 L). At this point, sodium chloride was added to assist in alleviating the emulsions and led to a 27-g dichloromethane fraction.

The 184-kg recollection from Sri Lanka was extracted with 280 L of methanol; the extract was divided between two 55-gallon steel containers and placed in the Tempe, Arizona, ambient temperatures from May 17 to June 26, 1989, to yield 9.86 kg (P-388 ED_{50} 6 μg/mL) of extract. A 1.3-kg aliquot of this methanol extract was subjected to solvent partitioning for the 196-g dichloromethane fraction, as described above, except that extraction of the 1:1 methanol−water solution by dichloromethane was followed by ethyl acetate to yield 29 g (P-388 − inactive) of hexane, 63 g (P-388 ED_{50} 20 μg/mL) of dichloromethane, and 46 g (P-388 inactive) of ethyl acetate fractions. Interestingly, the comparable dichloromethane fraction (27 g) from the Thai plant material (66.5 kg) processing provided a very close P-388 leukemia result (ED_{50} 19 μg/mL). In both cases, the remaining fractions from the solvent partitioning steps, including the residual aqueous fraction, were all inactive against the P-388 cell line. Consequently, all effort was focused on the dichloromethane fraction just described.

Isolation Procedures. The dichloromethane fraction (63 g) was passed through a Sephadex LH-20 column, using methanol-dichloromethane (2:3) as eluent. The second (fraction 1, 24.1 g) and third fractions (fraction 2, 33.1 g) were each rechromatographed on a Sephadex LH-20 column, using hexane−toluene−methanol (3:1:1) as eluent. The second fraction (fraction 3, 16.9 g) from the column chromatography of fraction 1 was chromatographed on a Si gel column with a gradient of hexane−dichloromethane as eluent. The first (64.4 mg) and second (13.6 mg) fractions eluted with hexane−dichloromethane (1:1) were subjected to HPLC [ODS, acetone−water (9:1)], affording schleicheol 2 (**9**) (15.6 mg) and schleicheol 1 (**8**) (3.4 mg), respectively. The third fraction (343 mg) eluted with hexane−dichloromethane (1:4) afforded sterol **10** (44.7 mg) after purification by HPLC [ODS, acetone−water (9:1)]. The third (fraction 4, 2.8 g) and the fifth (fraction 5, 2.4 g) fractions from the Sephadex LH-20 separation of fraction 2 were each repeatedly chromatographed on a Si gel column with a gradient of dichloromethane−methanol as eluent. The dichloromethane eluate (fraction 6, 46.8 mg) and the two fractions of dichloromethane−methanol (199:1) eluate (fraction 7, 102 mg; fraction 8, 171 mg) were collected from the fraction 4 chromatogram. The two fractions (fractions 6 and 8) and the other fraction (fraction 7) were purified by HPLC (ODS) using

9:1 and 17:3 methanol−water as eluents, respectively. Fractions 6, 7, and 8 gave schleicherastatin 2 (**2**) (5 mg); schleicherastatins 7 (**7**) (3 mg), 6 (**6**) (2.1 mg), and 5 (**5**) (10.2 mg); and schleicherastatins 4 (**4**) (3.9 mg), 3 (**3**) (6.3 mg), and 1 (**1**) (15.2 mg), respectively. The dichloromethane eluate from the Si gel chromatography of fraction 5 led to phenol **11** (65 mg).

Schleicherastatin 1 (1): recrystallization from methanol afforded needles; mp 185−187 °C; $[\alpha]_D$ +57° (*c* 0.4 $CHCl_3$); IR ν_{max} (KBr) 3440 (OH) cm^{-1}; HREIMS *m/z* 460.390 $[M]^+$ ($C_{30}H_{52}O_3$ 460.3919), 300.245 [M − CH_3O − $C_8H_{17}O$ (from the side chain)]$^+$ (calcd for $C_{21}H_{32}O$ 300.2450); ^1H and ^{13}C NMR data, measured at 500 and 127.5 MHz, respectively, see Table 1.

Crystal Structure of Schleicherastatin 1 (1). A colorless, rod-shaped crystal of sterol **1,** $C_{30}H_{52}O_3$, grown from a methanol solution, having approximate dimensions of 0.40 × 0.16 × 0.10 mm was mounted on a glass fiber with its long axis roughly parallel to the ϕ axis of the goniometer. Data collection was performed at 26 ± 1 °C using the $\omega/2\theta$ scan technique to a maximum 2θ of 140.0°. Cell constants and an orientation matrix for data collection were obtained from least-squares refinement, using the setting angles of 25 reflections in the range 10 to 40° θ, as measured by the computer-controlled diagonal slit method of centering. As a check on the crystal and electronic stability, three representative reflections were measured every 60 min during the collection. The slope of the least-squares line through a plot of intensity versus time was −11 counts $hour^{-1}$, which corresponded to a total loss in intensity of 6.8% over the total collection period. Crystal data: $C_{30}H_{52}O_3$, a = 11.475(2) Å, b = 15.397(3) Å, c = 16.373-(3) Å, V = 2893.9(10) Å^3, λ = 1.54178 A, ρ_c = 1.058 g cm^{-3}, ρ_o = 1.025 g cm^{-3} for Z = 4 and fw 460.72, $F(000)$ = 1024.

An octant of data (2978 reflections) was collected for sterol **1**. The systematic absences and subsequent statistical analysis of the complete reflection data set using the XPREP[13] program confirmed the space group as $P2_12_12_1$. A decay correction was made, and after Lorentz and polarization corrections, merging of equivalent reflections, and rejection of systematic absences, 2430 unique reflections remained, of which 1846 were considered observed [$I_o > 2\sigma(I_o)$] and used in the subsequent structure solution and refinement. An empirical absorption correction was also made to the data, based upon a series of ψ scans.[14] An extinction correction was not necessary. Direct methods structure determination and refinement were accomplished with the SHELXTL-V5.1.[13] package of programs. All non-hydrogen atoms for sterol **1** were located using the default settings of that program. The remaining hydrogen atom coordinates were calculated at optimal positions using the program SHELXL.[13] These atoms were assigned thermal parameters equal to either 1.2 or 1.5 (depending upon chemical type) of the U_{iso} value of the atom to which they were attached. Both coordinates and thermal values were forced to ride that atom during final cycles of refinement. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement process. The final standard residual R_1 value for the model shown in Figure 2 was 0.0817 (for observed data) and 0.1087 (for all data). The corresponding Sheldrick R values were wR_2 of 0.2067 and 0.2421, respectively. The difference Fourier map showed insignificant residual electron density; the largest difference peak and hole being +0.395 and −0.275 e/Å^3 , respectively. Final bond distances and angles were all within acceptable limits.[15]

Schleicherastatin 2 (2): needles from methanol; mp 185−187 °C; $[\alpha]_D$ −97° (*c* 0.2, $CHCl_3$); IR ν_{max} (KBr) 3440 (OH) cm^{-1}; HREIMS *m/z* 460.3919 $[M]^+$ (calcd for $C_{30}H_{32}O_3$, 460.3913), 300.2448 [M − CH_3O − $C_8H_{17}O$ (from the side chain)]$^+$ (calcd for $C_{21}H_{32}O$, 300.2450); ^1H and ^{13}C NMR assignments, see Tables 2 and 4.

Schleicherastatin 3 (3): obtained as needles from methanol; mp 178−180 °C; $[\alpha]_D$ +44° (*c* 0.3 $CHCl_3$); IR ν_{max} (KBr) 3252 (OH) cm^{-1}; HREIMS *m/z* 446.3758 $[M]^+$ (calcd for $C_{29}H_{50}O_3$: 446.3758), 300.2456 [M − CH_3O - $C_7H_{15}O$ (from the side chain)]$^+$ (calcd for $C_{21}H_{32}O$ 300.2450); ^1H and ^{13}C NMR data, measured at 300 and 75.4 MHz, respectively, see Tables 2 and 4.

Schleicherastatin 4 (4): needles from methanol; mp 148−151 °C; $[\alpha]_D$ −36° (*c* 0.2 $CHCl_3$); IR ν_{max} (KBr) 3451 (OH) cm^{-1}; HREIMS *m/z* 446.3757 $[M]^+$ (calcd for $C_{29}H_{50}O_3$, 446.3758), 300.2448 [M − CH_3O − $C_7H_{15}O$ (from the side chain)]$^+$ (calcd for $C_{21}H_{32}O$, 300.2452; ^1H and ^{13}C NMR data, see Tables 2 and 4.

Schleicherastatin 5 (5): needles; mp 162−166 °C; $[\alpha]_D$ −49° (*c* 1.0 $CHCl_3$); UV λ_{max} (ethanol) 237 nm (log ϵ 4.14); IR ν_{max} (KBr) 3425 (OH) cm^{-1}, 1666 (C═O) cm^{-1}; HREIMS *m/z* 444.3610 $[M]^+$ (calcd for$C_{29}H_{48}O_3$, 444.3601), 316.2400 [M − $C_8H_{16}O$ (from the side chain)]$^+$ (calcd for $C_{21}H_{32}O$, 316.2400); ^1H and ^{13}C NMR assignments, see Tables 2 and 4.

Schleicherastatin 6 (6): needles from methanol; 209−212 °C; $[\alpha]_D$ −94° (*c* 0.2 $CHCl_3$); UV λ_{max} (ethanol) 235 nm (log ϵ 3.79); IR ν_{max} (KBr) 3429 (OH), 1678 (C═O) cm^{-1}; HREIMS *m/z* 430.3447 $[M]^+$ (calcd for $C_{28}H_{46}O_3$, 430.3444), 316.2405 [M − $C_7H_{14}O$ (from the side chain)]$^+$ (calcd for $C_{21}H_{32}O$, 316.2400); ^1H and ^{13}C NMR data, see Tables 3 and 4.

Schleicherastatin 7 (7): isolated as needles following recrystallization from methanol; mp 148−152 °C; $[\alpha]_D$ −112° (*c* 0.1, $CHCl_3$); UV λ_{max} (ethanol) 235 nm (log ϵ 3.56); IR ν_{max} (KBr) 3424 (OH) cm^{-1}, 1674 (C═O); HREIMS *m/z* 430.3442 $[M]^+$ (calcd for $C_{28}H_{46}O_3$, 430.3444), 316.2400 [M − $C_7H_{14}O$ (from the side chain)]$^+$ (calcd for $C_{21}H_{32}O$, 316.2400); ^1H and ^{13}C NMR assignments, see Tables 3 and 4.

Schleicheol 1 (8): recrystallization from methanol gave needles; mp 82−88 °C; $[\alpha]_D$ +34° (*c* 0.7, $CHCl_3$); IR ν_{max} (KBr) 3410 (OH) cm^{-1}; HREIMS *m/z* 444.3959 $[M]^+$ (calcd for $C_{30}H_{52}O_2$, 444.3964); ^1H and ^{13}C NMR data, see Tables 3 and 4.

Schleicheol 2 (9): needles; mp 92−95 °C (from methanol); $[\alpha]_D$ −93° (*c* 1.7, $CHCl_3$); IR ν_{max} (KBr) 3452 (OH) cm^{-1}; HREIMS *m/z* 444.3980 $[M]^+$ (calcd for $C_{30}H_{52}O_2$, 444.3964); ^1H and ^{13}C NMR data, see Tables 3 and 4.

7-Oxo-*β*-Sitosterol (10): this previously known sterol recrystallized as needles from methanol; mp 120−123 °C; $[\alpha]_D$ −99° (*c* 2.2, $CHCl_3$); UV λ_{max} (ethanol) 237 nm (log ϵ 3.99); IR ν_{max} (KBr) 3450 (OH) cm^{-1}, 1674 (C═O); ^1H NMR ($CDCl_3$, 300 MHz) δ 0.64 (3H, s, H-18), 0.81 (3H, d, J = 6.8 Hz, H-26), 0.82 (3H, t, J = 6.8 Hz, H-29), 0.86 (3H, d, J = 6.8 Hz, H-27), 0.93 (3H, d, J = 6.8 Hz, H-21), 1.20 (3H, s, H-19), 2.04 (1H, dt, J = 12.7, 4.0 Hz, H-12β), 2.24 (1H, t, J = 11.0 Hz, H-8), 2.40 (1H, ddt, J = 13.0, 11.0, 1.8 Hz, H-4β), 2.51 (1H, ddd, J = 13.0, 5.0, 1.8 Hz, H-4α), 3.68 (1H, tt, J = 11.0, 5.0 Hz, H-3), 5.69 (1H, d, J = 1.8 Hz, H-6); ^{13}C NMR (75.4 MHz; $CDCl_3$) δ 12.0 (C-18 and C-29), 17.3 (C-19), 18.9 (C-21), 19.0 (C-26), 19.8 (C-27), 21.2 (C-11), 23.0 (C-28), 26.1 (C-23), 26.3 (C-15), 28.5 (C-16), 29.1 (C-25), 31.2 (C-2), 33.9 (C-22), 36.1 (C-20), 36.4 (C-1), 38.3 (C-10), 38.7 (C-12), 41.8 (C-4), 43.1 (C-13), 45.8 (C-8), 45.8 (C-24), 49.9 (C-9 and C-14), 54.7 (C-17), 70.5 (C-3), 126.1 (C-6), 165.2 (C-5), 202.4 (C-7); HREIMS *m/z* 428.3666 $[M]^+$ (calcd $C_{29}H_{48}O_2$, 428.3652).

Methyl protocatechuate (11): needles from toluene; mp 134−135 °C; EIMS *m/z* 168 $[M]^+$; identified by direct comparison with an authentic sample that was obtained by esterification (methanol, sulfuric acid) of protocatechuic acid.

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References and Notes

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