Novel Phloroglucinols from the Plant *Melicope sessiliflora* (Rutaceae)

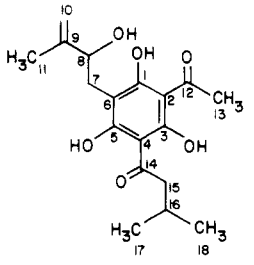
J. A. Chan,* E. A. Shultis, S. A. Carr, C. W. DeBrosse, D. S. Eggleston, T. A. Francis, L. J. Hyland, W. P. Johnson, L. B. Killmer, D. B. Staiger, and J. W. Westley

Research and Development, Smith Kline and French Laboratories, P.O. Box 1539, King of Prussia, Pennsylvania 19406-0939

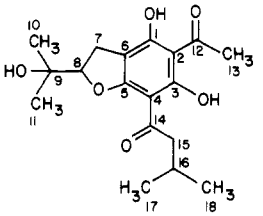
Received November 28, 1988

Hexane extraction of dried, pulverized leaves from the Australian plant *Melicope sessiliflora* followed by a bioassay-guided fractionation of the extract led to the isolation of three novel isomeric phloroglucinols with inhibitory activity against herpes simplex virus types I and II in vitro. The major active component, sessiliflorene, was shown to have the structure **1** by a combination of mass spectrometry and NMR methods. The two other bioactive isomers, sessiliflorols A and B, were resolved by HPLC whereupon the former crystallized. The structure of sessiliflorol A was determined as **2** by X-ray crystallography and the B isomer was shown to have structure **3** by spectroscopic comparison with **2**. Biosynthetic relationships between the three naturally occurring isomers are also proposed.

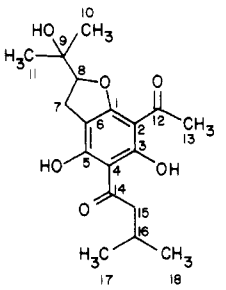
The genus *Melicope* (family Rutaceae) is known to produce a variety of compounds, including alkaloids,[1-3] triterpenoids,[1,4] flavanoids,[1,2,4] and sesquiterpenes.[4] As part of an ongoing natural product screening effort, a hexane extract of *Melicope sessiliflora* was found to inhibit growth of herpes simplex virus types 1 and 2 in vitro. An investigation of this hexane extract led to the isolation of three novel phloroglucinol-type compounds **1**, **2**, and **3**, which are the subject of this report.



1

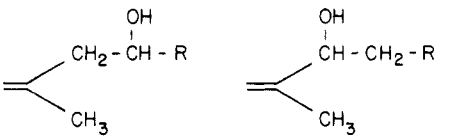


2



3

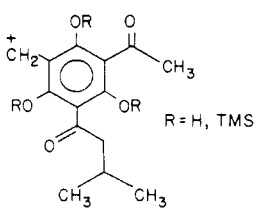
Sessiliflorene (**1**), an optically inactive compound by CD measurement, has the composition $C_{18}H_{24}O_6$ on the basis of high resolution EI mass spectrometry. Its IR spectrum indicates the presence of hydroxyl (3600–3100 cm^{-1}), hydrogen-bonded hydroxyl (3300–2400 cm^{-1}), and hydrogen-bonded carbonyl (1615 cm^{-1}) groups. The UV spectrum [MeOH λ_{max} 274 and 375 nm], which undergoes a bathochromic shift to 294 and 375 nm (infl) on addition of base, suggested the presence of a phenolic moiety. In fact, three phenolic hydroxyls were indicated by the ^1H NMR spectrum, which exhibited broad singlets at δ 16.20, 15.30 and 10.1, but showed no aromatic protons, suggesting a fully substituted benzene structure. The spin system at δ 0.98 [6 H, d, J = 6.7 Hz, $(CH_3)_2CH$], 2.25 [1 H, m, $(CH_3)_2CHCH_2$], and 3.05 [AMX, 2 H, $(CH_3)_2CHCH_2CO$] indicated an isovaleryl side chain and the connectivity of the protons was confirmed by 2D ^1H NMR spectroscopy. ^1H NMR spectroscopy also showed the presence of an acetyl side chain at δ 2.72 (3 H, s, CH_3CO). ^{13}C NMR spectroscopy showed six quaternary carbons, three at high field (δ 104.3) and three at low field (δ 165.5–170.5), consistent with a fully substituted, 1,3,5-trioxygenated aromatic nucleus. The structure of the remaining side chain was deduced in the following manner. ^{13}C NMR spectroscopy showed the presence of a methyl at δ 18.5, a methylene at δ 112.9, suggesting a vinyl carbon and a second methylene at δ 53.0, one downfield methine carbon with an oxygen substituent at δ 77.7 and a quaternary vinyl carbon at δ 145.3. 2D ^1H NMR spectroscopy indicated that the methine hydrogen (δ 4.37) is coupled to the methylene hydrogens (δ 3.11, 2.75), while the methyl hydrogens (δ 1.86) are coupled to the terminal vinyl hydrogens (δ 5.00 and 4.90). The partial structure of this side chain could therefore be either A or B:



A B

Partial structure B was supported by a proton homonuclear correlated experiment modified to observe long-range couplings, which indicated that the methine hydrogen at δ 4.37 and the terminal vinyl hydrogens at δ 5.00 and 4.90 were also coupled.

Finally, a mass spectral base peak fragment corresponding to the loss of the isobutenyl alcohol moiety by allylic cleavage to yield fragment **4** was observed in the EI (Table I) and CH_4-CI mass spectra of **1** and its tetra TMS



R = H, TMS

4

(1) Murphy, S. T.; Ritchie, E.; Taylor, W. C. *Aust. J. Chem.* **1976**, *29*, 187.
(2) Fauvel, M. T.; Gleye, J.; Moulis, C.; Blasco, F.; Stanislas, E. *Phytochemistry* **1981**, *20*, 2059.
(3) Ahond, A.; Picot, F.; Potier, P.; Poupat, C.; Severet, T. *Phytochemistry* **1978**, *17*, 166.
(4) Free, A. J.; Read, R. W.; Ritchie, E.; Taylor, W. C. *Aust. J. Chem.* **1976**, *29*, 695.

0022-3263/89/1954-2098$01.50/0

Table I. Abbreviated EI Mass Spectral Data of Compounds 1, 2, 3, 5, 6, 7, and 8

m/z	1	2	3	5	6	7	8
43	31	[c]					
57	6	13	20	18	15	17	17
59	2	39	31	10	12	11	8
67	7	7	9	10	10	10	13
69	8	14	14	17	15	33	23
77	4	8	15	8	10	19	13
207	3	36	34	56	44	10	14
219	3	11	11	6	11	15	21
221		19	11	4	6		
234						10	17
243							11
247	16	7	8	17	20	7	12
249		14	11	30	29		
252		3	12	16	16		
261		14	15	18	14	75	100
263		24	14	19	31	25	41
265	100[a]	5	13	63	62		
277		34	16				
278		24	11	2			
279		32	80	80	53		
294		3	7	4	8		
303	3	30	22	17	27	65	44
318	25			2	5	100[a]	94[a]
319		1	16	2	8		
321		62	37	20	16		
336	1[b]	100[b]	100[b]	100[b]	100[b]		

[a] Fragment 4 (R = H). [b] M^+. [c] Blank indicates less than 1% relative abundance.

derivative. These data rule out partial structure A and together with the NMR data above unequivocally confirm the structure of sessiliflorene to be 1.

Sessiliflorol A (2) and sessiliflorol B (3) have similar UV spectra and an identical molecular formula to that of sessiliflorene (1). They are both optically inactive on the basis of CD measurements. The ^1H NMR and ^{13}C NMR spectra showed the presence of acetyl and isovaleryl side chains very similar to that of sessiliflorene, 1. These features were also confirmed in the EI MS data by the observed losses of CH_2CO, C_4H_9, and C_4H_9CO from the molecular ion (Scheme I, Table I). The only differences between the sessiliflorols and sessiliflorene are in the remaining side chains and the absence of the phenolic proton at δ 10.1. The 2D ^1H NMR spectrum of sessiliflorol B (3) indicated coupling between a methylene (δ 3.08, 2 H, AMX, J = 9.3, 15.1 Hz) and a methine hydrogen (δ 4.84, 1 H, t, J = 9.3 Hz). The ^{13}C NMR (GASPE) spectrum showed the presence of a methylene carbon (δ 51.4), a methine carbon with an unusual downfield chemical shift at δ 92.9, a quaternary carbon at δ 71.7, and two methyls at δ 24.9 and 24.7. The base peak in the EI mass spectra of 2 and 3 is the molecular ion at m/z 336. This fact, together with the considerably decreased intensity of the $(M - 71)^+$ peak in the EI mass spectra of 2 and 3 compared to that of 1, suggested that the allylic cleavage was no longer operative and the isobutenyl alcohol moiety had been incorporated into a more stable ring structure. The presence of an α-(hydroxyisopropyl)dihydrofuran moiety is substantiated by the strong peak at m/z 59 and the previously observed[5] rearrangement and loss of isobutene oxide at m/z 279 in the EI mass spectrum. ^1H NMR spectroscopy of the peracetylated derivatives of 2 and 3 showed that the methine hydrogen at δ 4.84 (H-8) was shifted by 0.3 ppm downfield to δ 5.10, suggesting the presence of a secondary alcohol. All of the above data indicate that the structures of the two sessiliflorols are 2 and 3. The two compounds

(5) Carpenter, I.; McGarry, E. J.; Sheinmann, F. *Tetrahedron Lett.* **1970**, 3983.

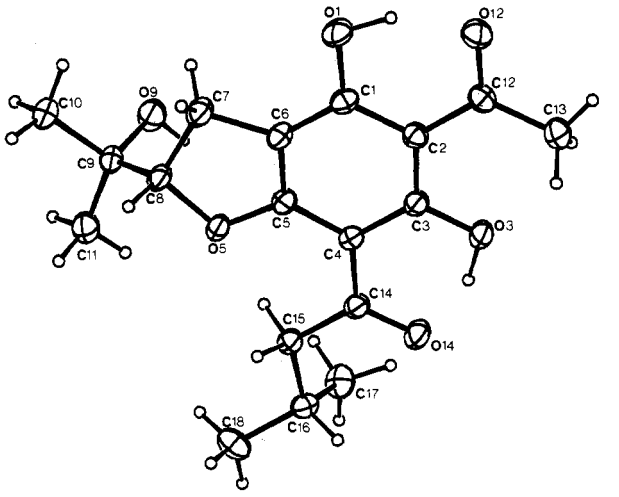
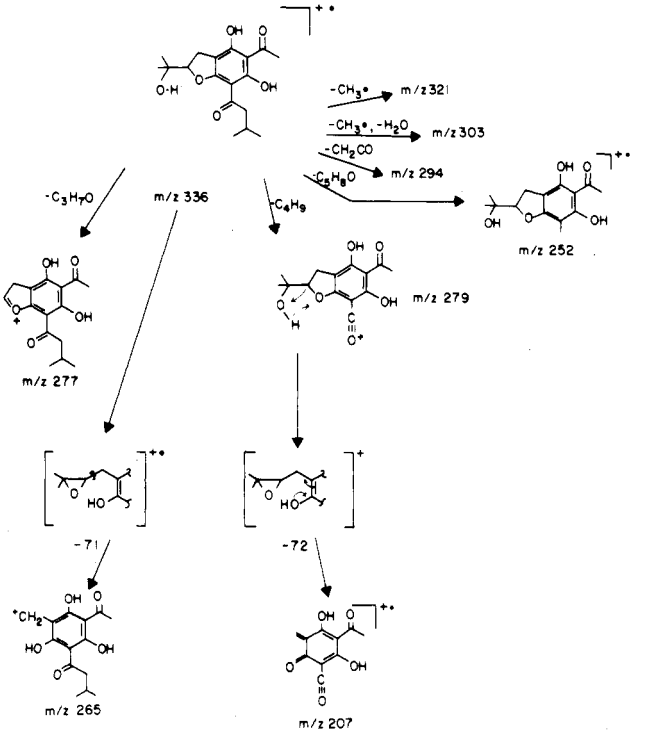


Figure 1. ORTEP drawing of sessiliflorol A (2) with numbering; thermal ellipsoids are drawn at the 50% probability level.

Scheme I. EI-MS Fragmentation Pattern of Sessiliforol A (2)



were distinguished by single crystal X-ray diffraction of 2 (Figure 1).

An attempt to confirm the structures of 2 and 3 by acid-catalyzed cyclization of sessiliflorene gave two major sets of isomeric products (see Scheme II), neither of which corresponded to the sessiliflorols. The structures of the cyclized products were elucidated on the basis of UV, MS (Table I), ^{13}C NMR (Table II), and ^1H NMR (Tables V and VI) data.

Compounds 5 and 6 were indistinguishable based on molecular weight (m/z 336), MS fragmentation pattern (Table I), ^1H NMR (Table V) and ^{13}C NMR (Table II) data and UV profile, except for the UV extinction coefficient at λ_{max} 273 nm and the HPLC retention time. The ^1H and ^{13}C NMR spectra of the two isomers showed the presence of both the acetyl and isovaleryl side chains. The structure of the remaining side chain of compounds 5 and 6 was deduced in the following manner. ^{13}C NMR spectra

Table II. ^{13}C NMR Spectral Data for Compounds 1, 2, 3, 5, 6, and 7 in $CDCl_3$

C	1	2	3	5	6	7
12, 14	206.9	204.5	207.0	204.4	206.9	204.8
	204.4	204.4	202.0	203.9	202.5	204.5
	170.5	171.3	170.7	170.5	173.0	171.5
1, 3, 5	169.5	166.7	170.0	169.6	170.0	166.9
	165.5	166.3	166.8	167.5	170.0	166.3
	104.3	105.3	105.2	104.1	105.5	*a*
2, 4, 6	104.3	104.4	104.6	*a*	103.0	*a*
	104.3	100.3	100.4	98.0	98.0	*a*
13	33.0	33.0	31.1	32.9	33.1	33.0
15	28.4	26.2	26.3	25.6	25.4	25.7
16	25.3	26.1	26.0	25.4	25.1	25.4
17, 18	22.8	22.7	22.7	22.7	22.8	22.7
7	53.0	52.9	51.4	53.2	53.0	51.3
8	77.7	92.8	92.9	79.9	80.1	80.2
9	145.3	71.5	71.7	68.4	68.4	145.3
10	112.9	25.5	24.9	25.1	25.1	112.9
11	18.5	24.7	24.7	25.1	25.1	17.1

[a] Peak intensities were too weak to determine chemical shifts accurately.

Table III. ^1H NMR Spectral Data for Compound 1 in $CDCl_3$

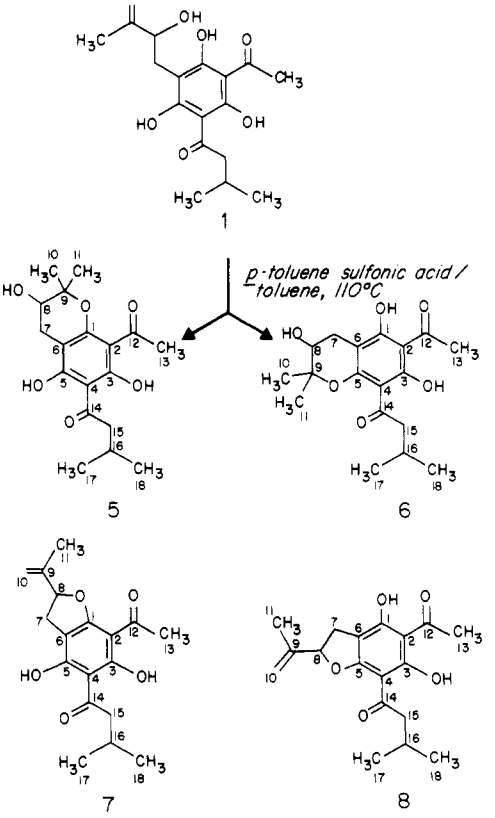
C	chem shift	intgrtn	mult	*J*, Hz	assgnmt
1	16.2	1	s		OH[a]
3	15.3	1	s		OH[a]
5	10.1	1	s		OH[a]
7a,b	3.11	1	dd	15.5	CH_2CH
	2.75	1	dd	15.5, 7.3	
8	4.37	1	d	7.3	CH–O
10a,b	5.00	1	s (br)	0	CH_2
	4.90	1	s (br)	0	
13	2.72	3	s (br)		$CH_3C{=}CH_2$
11	1.86	3	s		CH_3CO
15	3.05	2	AMX		$COCH_2CH$
16	2.25	1	m	6.7, –, –	$CH_2CH(CH_3)_2$
17, 18	0.98	6	d	6.7	$(CH_3)_2CH$

[a] Doubling of the OH peaks was observed due to slow interconversion between the two bis-hydrogen-bonded structures. This was confirmed by a spin-saturation transfer experiment.

showed the presence of an oxygen-bearing methine at δ 79.9 in one case and δ 80.1 in the other, suggesting a dihydropyran in contrast to compound **2**, which showed the presence of an oxygen-bearing methine at δ 92.8, consistent with the dihydrofuran present in sessiliflorol A. The six-membered ring structure for compounds **5** and **6** was supported further by the presence in the ^1H NMR spectrum of an oxygen-bearing methine at δ 3.86 (t, J = 5.3 Hz), in contrast to that of compound **2** which showed the presence of an oxygen-bearing methine at δ 4.83 (t, J = 9.0 Hz). The oxygen-bearing methine at δ 3.86 was coupled to a methylene at δ 2.92 in one case and δ 2.99 (AMX, J_X = 5.3 Hz) in the other, and the foregoing data together with the two methyl singlets at δ 1.46, 1.44 and δ 1.42, 1.41 suggested the structure of compounds **5** and **6** as shown. The mass spectra of compounds **5** and **6** (Table I) differ from those of **2** and **3** in exhibiting a major fragment ion at m/z 265, $(M - 71)^+$. Collisional activation of the protonation parent (m/z 337) produced by FAB ionization of **5** or **6** and analysis of the resulting daughter ions in a triple quadrupole mass analyzer[6] yield spectra with the base peak at m/z 265. This indicates that the fragment is formed directly from the molecular species, possibly by a mechanism analogous to that illustrated for compound **2** in Scheme I. As noted earlier, compound **2** was distinguished from compound **3** by X-ray crystallographic studies.

(6) McLafferty, F. W. *Science* **1981**, *214*, 280.

Scheme II. Acid-Catalyzed Cyclization Products of Sessiliflorene (1)



Compound **3**, which is more polar than compound **2** based on chromatographic data, also gave a higher (×1.68) extinction coefficient at λ_{max} 273 nm. By analogy, compound **5** is assigned the structure shown because it is both more polar than compound **6** and also has a higher extinction coefficient (×1.66) at λ_{max} 274 nm.

Compounds **7** and **8** were indistinguishable in their molecular weight (m/z 318), MS fragmentation pattern (Table I), ^1H NMR data (Table VI), and UV profile except for the extinction coefficient at λ_{max} 273 nm and HPLC retention time. The mass spectra of compounds **7** and **8** yielded intense molecular ions at m/z 318, suggesting that they are dehydration products of compounds **2** and **3** (m/z 336). The fragmentation pattern of compounds **7** and **8** is very similar to that observed for compounds **2** and **3** with the exception of a higher relative abundance for the fragment ion at m/z 261, which arises presumably from the α-cleavage of the isobutenyl group. The dehydration that formally distinguishes **7** and **8** from the sessiliforols occurs in the hydroxyisopropyl side chain, as suggested by the presence of a terminal vinyl methylene at δ 112.9 and a quaternary vinyl carbon at δ 145.3 in the ^{13}C NMR spectra of the two synthetic compounds. This proposal is supported by ^1H NMR spectroscopy with the appearance of two terminal vinyl hydrogens at δ 4.96, 4.94 and δ 5.07, 5.05 and by a methyl attached to a vinyl carbon at δ 1.76. On the basis of this spectroscopic data, the structure of compounds **7** and **8** is as shown. Following the same empirical argument as that proposed above for the sessiliflorols and compounds **5** and **6**, compound **7** is assigned as shown because it is both more polar than compound **8** and has a higher extinction coefficient (×2.80) at λ_{max} 272 nm. Further work to confirm these assignments is underway.

Acylphloroglucinol derivatives are quite common in nature and Birch[7] has demonstrated that they usually

Table IV. ^{1}H NMR Spectral Data for Compounds 2 and 3 in $CDCl_3$

	chem shift		intgrtn	mult		J, Hz		assgnmt	
C	2	3	2 or 3	2	3	2	3	2	3
1	15.8		1	s				OH	
3	14.7	14.9	1	s	s			OH	OH[a]
5		15.6	1		s				OH[a]
7a,b	3.08	3.07	2	AMX	AMX	9.0, 14.1	9.3, 15.1	CH_2CH	
8	4.83	4.84	1	t	t	9.0	9.3	$CHCH_2$	
10	1.39	1.38	3	s	s			$HOC(CH_3)_2$	
11	1.28	1.27	3	s					
13	2.70	2.64	3	s	s			CH_3CO	
	2.93		1	dd		6.9, 14.5			
15a,b		2.97			AMX		6.9, 21.1[b]	$COCH_2CH$	
	2.81		1	dd		6.9, 14.5			
16	2.20	2.25	1	m	m		6.6[b]	$CH_2CH(CH_3)_2$	
17	0.99	0.97	6	d	d		6.7	$(CH_3)_2CH$	
18									

[a] Distinguished on basis of long-range coupling between hydrogens 5 and 15. [b] Apparent coupling constants.

Table V. ^{1}H NMR Spectral Data for Compounds 5 and 6 in $CDCl_3$

	chem shift		intgrtn	mult	J, Hz		assgnmt
C	5	6	5 or 6	5 or 6	5	6	5 or 6
1		16.1	1	s			OH
3	16.2	15.4	1	s			OH
5	15.4		1	s			OH
7a,b	2.92	2.99	2	AMX		J_X 5.3	CH_2CH
8	3.86	3.86	1	t	5.3	5.3	$CHOHCH_2$
10	1.46	1.44	3	s			$(CH_3)_2CO$
11	1.42	1.41	3	s			CH_3CO
13	2.72	2.65	3	s			
15a,b	2.88	2.87	1	dd	5.3, 9.8	5.0, 17.2	$COCH_2CH$
	2.64	2.63	1	dd			
16	2.21	2.25	1	m	6.4, 5.0	6.6, 5.0	$CH_2CH(CH_3)_2$
17, 18	0.98	0.97	6	d	6.4	6.6	$(CH_3)_2CH$

Table VI. ^{1}H NMR Spectral Data for Compounds 7 and 8 in $CDCl_3$

	chem shift		intgrtn	mult		J, Hz	assgnmt
C	7	8	7 or 8	7 or 8	7	8	7 or 8
1		15.6	1	s			OH
3	15.8	14.9	1	s			OH
5	14.7		1	s			OH
7a,b	3.24	3.25	1	dd	15.0, 9.0	14.9, 8.5	CH_2CH
	2.90	2.89	1	dd			
8	5.37	5.37	1	t	9.0	8.5	$CHCH_2$
10	5.07	5.05	1	s (br)			CH_2
10	4.96	4.94	1	s (br)			
11	1.76	1.76	3	s			CH_3CH_2
13	2.68	2.61					$CH_3C{=}O$
15a,b	2.84	2.95	2	AMX			$COCH_2CH$
16	2.16	2.18	1	m			$CH_2CH(CH_3)_2$
17, 18	0.96	0.95	6	d	6.6	6.6	$(CH_3)_2CH$

originate from acetate via a polyketide precursor. Presumably, once the *C*-acetylphloroglucinol chromophore is formed, two ring prenylations lead to the basic sessiliflorane carbon skeleton. In the foregoing discussion, summarized in Scheme II, the major products of the acid-catalyzed rearrangement of sessiliflorene were established as the bicyclic pairs of isomers, **5** and **6**, **7** and **8**, rather than the naturally occurring alcohols, sessiliflorol A (**2**) and B (**3**). This leads to the hypothesis that rather than the alcohols being biosynthetically derived from sessiliflorene, all three natural products could be derived from a single precursor, such as the epoxide **9**, proposed in Scheme III. This compound in turn could be the result of epoxidation of the dimethylallyl precursor formed on prenylation of the chromophoric acylphloroglucinol. The proposed epoxide precursor **9** then could isomerize by route a to sessiliflorene or cyclize via routes b or c to sessiliflorols A and B, respectively. The reason for the lack of optical activity in these natural phloroglucinols is not clear at this time.

Table VII. In Vitro Antiherpes Activity of Phloroglucinols 1, 2, and 3

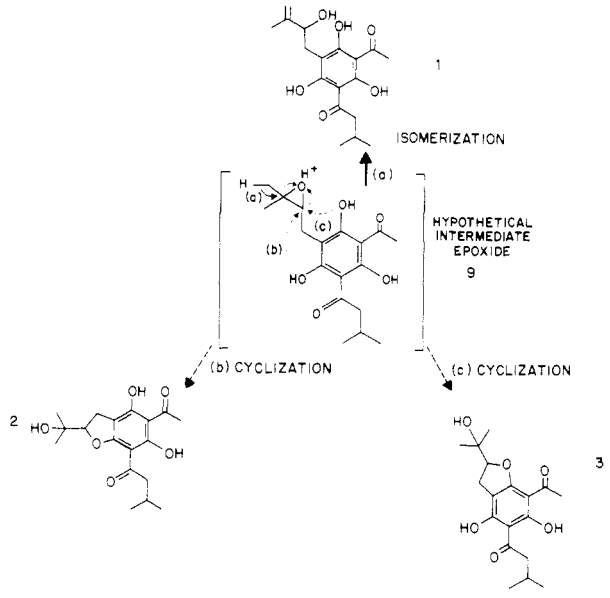
		IC_{50}, μM	
compd	name	HSV-1	HSV-2
1	sessiliflorene	0.3	3.0
2	sessiliflorol A	22.3	10.4
3	sessiliflorol B	44.6	10.4
control	acyclovir	1.2	0.5

Confirmation of the isomerization of the proposed precursor to sessiliflorene epoxide awaits the isolation or synthesis of **9**; however, there are many precedents reported for the prenyl group cyclizations to the dihydrobenzofuran chromophore in coumarins,[8,9] rotenoids,[10]

(7) Birch, A. J. *Proc. Chem. Soc.* **1962**, 3.

(8) Gray, A. I.; Waterman, P. G. *Phytochemistry* **1978**, *17*, 845.
(9) McHale, D.; Khopkar, P. P.; Sheridan, J. B. *Phytochemistry* **1987**, *26*, 2547.

Scheme III. Proposed Biosynthetic Relationship between Sessiliflorene (1), Sessiliflorol A (2), and Sessiliflorol B (3)



chromones,[11] and flavanoids.[12]

The antiherpes activity, in vitro, of these compounds is summarized in Table VII. The design of similar molecules to enhance antiherpes potency (IC_{50} < 1 μM) with HSV-1 and HSV-2 is underway and a number of potent analogues have already been synthesized. This work will be the subject of a future publication. Under our assay conditions, all of the compounds were cytotoxic to Vero cells, but doses of 35 μM to 45 μM were required to achieve a 50% reduction in thymidine or leucine incorporation. To date, the compounds have failed to exhibit in vivo activity.

Experimental Section

General Methods. Melting points were obtained on a Kofler hot stage microscope apparatus and are uncorrected. CD spectra were recorded on a JASCO J-500C spectrometer, using acetonitrile as solvent. UV spectra were measured on a Beckman DU-7 instrument. IR spectra were recorded on a Nicolet 20DXB FTIR as methylene chloride film. ^1H NMR spectra were measured on either a Bruker Instruments WM360 or a JEOL GX270 spectrometer, operating at 360.13 MHz and 270.05 MHz, respectively, maintained at 19 °C. ^{13}C NMR spectra were measured on the same instruments operating at 90.56 MHz or 67.51 MHz, respectively. The samples, ca. 20 mg, were dissolved in $CDCl_3$ (MSD, 99.96% D) for analyses. Edited ^{13}C spectra were measured using a *GATED SP*in *E*cho pulse sequence. Two-dimensional ^1H COSY spectra were measured by using the standard pulse sequences, ^1H spectral windows were expanded to 20 ppm to accommodate the downfield shift of the H-bonded phenolic protons. Electron impact mass spectra were obtained with a Varian Mat CH5-DF mass spectrometer equipped with an EI/FI/FD combination source by direct insertion of the samples into a heated ion source. Ions were produced at a probe temperature of 160 °C, a source temperature of 260 °C, and an ionization energy of 70 eV. Perfluorokerosene, Lot H, SCM Specialty Chemicals (PFKH), was used to calibrate the mass range of m/z 30 to m/z 831. The spectra were recorded and processed by an Incos 2000 data system. Approximately 200 μL of CH_2Cl_2 was added to each sample vial containing the compound isolated from *M. sessiliflora*. Aliquots of 2–4 μL were loaded on the probe tip. High resolution exact mass measurements were done later by peak matching of the molecular species to the closest down field reference peak of PFKH at a resolution of >5000 (5% valley). All values were within 3 amu of the theoretical elemental composition. HPLC analyses were monitored by using either an ISCO V4 absorbance detector or Spectra Monitor III, Model #1204A.

Antiherpes Virus Bioassay. The bioassay for antiherpes activity with HSV-1 and HSV-2 has been described.[13] Briefly, Vero cells in culture are exposed continuously to drug in the growth medium for 48 h after a 1-h challenge with virus. The amount of cytopathic effect (cpe; characteristic of infection by each viral strain) present was scored visually. Results were expressed as percent cpe relative to controls and IC_{50} values are reported as the concentration of material required to produce a 50% reduction in cpe.

Extraction and Separation of Bioactive Components. *M. sessiliflora* was collected in Australia in September, 1979, and was identified by C. T. White; voucher specimen VKM 2666 is preserved in National Herbarium, Washington, DC. Dry pulverized leaves from *M. sessiliflora* (30 g) were stirred in 1.2 L of *n*-hexane for 24 h. Filtration and concentration of the filtrate under vacuum yielded 650 mg of crude extract, which was chromatographed on silica gel 60 (EM Reagents, 230–400 mesh) on an Altex column (25 mm × 1000 mm). Elution was performed by using sequentially CH_2Cl_2, 0.2% H_2O/1% CH_3OH/CH_2Cl_2, and 0.2% H_2O/2% CH_3OH/CH_2Cl_2 (40–80 psi using a Beckman 112 HPLC pump with preparative head) while monitoring at 272 nm. The first bioactive fraction, containing compound **1** (42 mg), was obtained in the CH_2Cl_2 eluate. Recrystallization from Et_2O/hexane gave 32 mg of white crystals. A second major bioactive fraction (226 mg) was eluted with 0.2% H_2O/1% CH_3OH/CH_2Cl_2. This fraction was further resolved by reversed phase chromatography on Whatman ODS-3 (10 μm, 25 mm × 500 mm), using 70% CH_3OH in H_2O as eluant (160–190 psi, using a Beckman 112 HPLC pump with preparative head) and monitoring at 272 nm. The major bioactive fraction from this second chromatography consisted of 100 mg of a mixture of **2** and **3**. This mixture was separated by multiple injections on a Merck Lichorosorb silica gel column (7 μm, 10 mm × 250 mm) with 0.2% H_2O/1% CH_3OH/CH_2Cl_2 as eluant at 4 mL per min (450 psi using a Consta Metric III pump) and monitoring at 272 nm. Compound **2** (33 mg) eluted at 7.5 min and **3** (40 mg) eluted at 8.4 min. Compound **2** was crystallized from Et_2O/hexane.

Compound **1**: IR (CH_2Cl_2 film) 3600–3100 (OH), 3300–2400 (hydrogen-bonded OH), 1615 cm^{-1} (hydrogen-bonded carbonyl); UV (MeOH) λ_{max} (ε) 274 (37 529), 375 nm (4941); UV (MeOH–0.1 N NaOH) 295 (39 059), 375 nm (infl); calcd for $C_{18}H_{24}O_6$ 336.1572, found M^+ m/z 336.1557; mp 117–120 °C. Anal. Calcd for $C_{18}H_{24}O_6$: C, 64.27; H, 7.19. Found: C, 64.52; H, 7.33; MS fragments, see Table I; ^1H NMR, see Table III; ^{13}C NMR, see Table II.

Compound **2**: IR (CH_2Cl_2 film) 3600–3100 (OH), 3300–2400 (hydrogen-bonded OH), 1636 cm^{-1} (hydrogen-bonded carbonyl); UV (MeOH) λ_{max} (ε) 273 (39 969), 341 nm (3737); UV (MeOH–0.1 N NaOH) 293 (36 969), 320 nm (infl); calcd for $C_{18}H_{24}O_6$ 336.1572, found M^+ m/z 336.1564; mp 105–107 °C; MS fragments, see Table I; ^1H NMR, see Table IV; ^{13}C NMR, see Table II.

Compound **3**: IR (CH_2Cl_2 film) 3600–3100 (OH), 3200–2400 (hydrogen-bonded OH), 1633 cm^{-1} (hydrogen-bonded carbonyl); UV (MeOH) λ_{max} (ε) 273 (65 999), 341 nm (8148); UV (MeOH–0.1 N NaOH) 294 (48 451), 320 nm (infl); calcd for $C_{18}H_{24}O_6$ 336.1572, found M^+ m/z 336.1571; MS fragments, see Table I; ^1H NMR, see Table IV; ^{13}C NMR, see Table II.

Cyclization of Compound 1 and Separation of Cyclization Products. The major active component, **1** (40 mg), was heated with *p*-toluenesulfonic acid (40 mg) in toluene (6 mL) at 90–100 °C under a nitrogen atmosphere. After 2.5 h, the mixture was concentrated, taken up in Et_2O (25 mL), and washed with aqueous $NaHCO_3$ (1% w/v; 4 × 20 mL), and the Et_2O layer was washed with H_2O (4 × 20 mL) and dried over $MgSO_4$. Evaporation of the solvent under reduced pressure gave a mixture (40 mg). This mixture was separated by multiple injections (4 mg each) on an HPLC preparative scale reverse phase column (Magnum 9, 250 mm × 10 mm, Partisil 10 μm, ODS-3) with 0.1% TFA/10%

(10) Crombie, L. *Nat. Prod. Rep.* **1984**, 3.
(11) Gray, A. I. In *Chemistry and Chemical Taxonomy of the Rutales*; Waterman, P. G., Grundon, M. F., Eds.; Academic Press: London, 1983; Chapter 4.
(12) Roussis, V.; Ampofo, S. A.; Wirmev, D. F. *Phytochemistry* **1987**, *26*, 2371.
(13) Poehland, B. L.; Carte, B. K.; Francis, T. A.; Hyland, L. J.; Allaudeen, H. S.; Troupe, N. *J. Nat. Prod.* **1987**, *50*, 706.

H_2O/CH_3OH as eluant at 5 mL per min (3.4 Kpsi). A mixture (6.4 mg) of **5** and **6** eluted as the major peak at 6.6 min, and a mixture (5.5 mg) of **7** and **8** eluted as a peak at about 10 min. Separation of **4** and **5** was carried out on the same Magnum 9 column (above), eluting with 0.1% TFA/35% H_2O/CH_3OH at 5 mL per min (3.3 Kpsi). Compound **5** (1.9 mg) eluted at about 32 min and **6** (2.8 mg) eluted at about 36 min. Separation of **7** and **8** was carried out on a Magnum 9 column, eluting with 0.1% TFA/20% H_2O/CH_3OH at 5 mL per min (3.7 Kpsi). Compound **7** eluted at 33 min and **8** eluted at 37 min.

Compound **5**: IR (CH_2Cl_2 film) 3600–3100 (OH), 3200–2300 (hydrogen-bonded OH), 1616 cm^{-1} (hydrogen-bonded carbonyl); UV (MeOH) λ_{max} (ϵ) 274 (48 754), 340 nm (5724); calcd for $C_{18}H_{24}O_6$ 336.1572, found M^+ m/z 336.1567; MS fragments, see Table I; ^1H NMR, see Table V, ^{13}C NMR, see Table II.

Compound **6**: IR (CH_2Cl_2 film) 3600–3100 (OH), 3200–2300 (hydrogen-bonded OH), 1615 cm^{-1} (hydrogen-bonded carbonyl); UV (MeOH) λ_{max} (ϵ) 274 (28 889), 338 nm (2660); calcd for $C_{18}H_{24}O_6$ 336.1572, found M^+ m/z 336.1564; MS fragments, see Table I; ^1H NMR, see Table V, ^{13}C NMR, see Table II.

Compound **7**: IR (CH_2Cl_2 film) 3600–3100 (OH), 3300–2400 (hydrogen-bonded OH), 1634 cm^{-1} (hydrogen-bonded carbonyl); UV (MeOH) λ_{max} (ϵ) 272 (28 860), 341 nm (2962); calcd for $C_{18}H_{22}O_5$ 318.1467, found M^+ m/z 318.1464; MS fragments, see Table I; ^1H NMR, see Table VI, ^{13}C NMR, see Table II.

Compound **8**: IR (CH_2Cl_2 film) 3600–3100 (OH), 1631 cm^{-1} (hydrogen-bonded carbonyl); UV (MeOH) λ_{max} (ϵ) 272 (10 089), 335 nm (2146); calcd for $C_{18}H_{22}O_5$ 318.1467, found M^+ m/z 318.1455; MS fragments, see Table I; ^1H NMR, see Table VI.

Acetylation of 2 and 3. A 15-mg mixture of **2** and **3** was dissolved in 2 mL of Ac_2O (Supelco, freshly opened ampule) and stirred at 110 °C under N_2 atmosphere for 24 h. The reaction mixture was concentrated under reduced pressure, stirred for 1 h at room temperature with aqueous Na_2CO_3 (4 mL, 10% w/v), and then extracted with Et_2O (3 × 4 mL). The Et_2O layer was washed with H_2O (4 mL), combined, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give 15 mg of a mixture of acetylated product. The mixture was separated by HPLC, using multiple injections of about 2 mg each on a Merck Lichrosorb column (7 μm, 10 mm × 250 mm) and elution with 0.2% H_2O/1% CH_3OH/CH_2Cl_2 at 3 mL/min (Consta Metric III Pump), monitored at 325 nm. Chromatography yielded major peaks at 7.0 min (9.2 mg), 15.9 min (3 mg), and 18.5 min (2.5 mg).

The major peak at 7.0 min was identified as the triacetylated product of compounds **2** and **3** on the basis of the following spectroscopic data: MS m/z 462 (M^+), 420 ($M - CH_2CO$), 360 ($420 - CH_3CO_2H$), 318 ($360 - CH_2CO$), 303 ($318 - CH_3$); ^1H NMR 0.94 (d, 6 H, $(CH_3)_2CH$), 1.55 (bs, 6 H, $(CH_3)_2COAc$), 1.98, 2.26, 2.28 (s, 9 H, OAc), 2.55 (s, 3 H, CH_3CO), 2.57, 2.78 (dd, 2 H, J = 6.7 Hz, H_{15a}, H_{15b}), 3.08 (ABX, 2 H, H_{7a}, H_{7b} J = 9.0, 14.5 Hz), 5.12 ppm (t, 1 H, J = 8.0 Hz, H-8); IR (CH_2Cl_2 film), 1777 (carbonyl aryl ester), 1738 (carbonyl aliphatic ester), 1695 cm^{-1} (aryl ketone); UV (MeOH) λ_{max} (ϵ) 241 (15 819), 307 nm (3935).

The peak at 15.7 min was identified as the diacetylated phenolic product of compounds **2** and **3** on the basis of the following spectroscopic data: m/z 420 (M^+); ^1H NMR 0.95 (d, 6 H, $(CH_3)_2CH$), 1.25, 1.36 (s, 6 H, $(CH_3)_2COH$), 2.24 (m, 1 H, H-16), 2.28, 2.25, (s, 6 H, OAc), 2.40, 2.78 (dd, 2 H, H_{15a}, H_{15b}), 2.41 (s, 3 H, CH_3CO), 3.08 (m, 2 H, H_{7a}, H7b), 4.80 ppm (t, 1 H, J = 9.3 Hz, H-8); IR (CH_2Cl_2 film), 1775 (carbonyl aryl ester), 1692 cm^{-1} (carbonyl aryl ketone); UV (MeOH) λ_{max} (ϵ) 241 (11 849), 305 nm (2718).

The peak at 18.5 min was identified as the diacetylated phenolic product of compounds **2** and **3**. The MS, ^1H NMR, IR, and UV data were similar to those for the peak at 15.7 min.

Single-Crystal X-ray Diffraction Analysis of Compound 2. Single-crystal X-ray diffraction analysis of **2** was carried out from three-dimensional intensity data collected on an Enraf-Nonius CAD-4 diffractometer [λ(Mo Kα) = 0.71073 Å] equipped with a graphite monochromator. Data were collected by a variable speed 2θ scan technique at 248 K. A total of 5583 data ($\pm h$, $+k$, $+l$) were collected ($2\theta \leq 60°$), of which 3623 were considered observed ($I \geq 3\sigma(I)$) after correction for Lorentz and polarization effects and after averaging symmetry equivalent reflections (R_{int} = 0.013). The molecule crystallizes as a hydrate from CH_3OH. Crystal data: triclinic, $P\bar{1}$, Z = 2, a = 9.852 (2), b = 11.563 (2), and c = 8.931 (2) Å, α = 107.51 (2)°, β = 110.54 (2)°, γ = 73.36 (2)°. The structure was solved by direct methods. Non-hydrogen atoms were refined with anisotropic vibrational parameters; all hydrogens were located from difference Fourier syntheses and were refined with isotropic temperature factors. Full matrix least-squares refinement (on F) converged to values of the standard crystallographic residuals, R = 0.043 and R_w = 0.055. An extinction coefficient refined to 1.24 (1) × 10^{-6}. The final difference Fourier map was featureless. Additional data have been deposited as supplementary material.

Acknowledgment. We thank Professor Sidney M. Hecht of the University of Virginia for supplying the plant material and also for helpful discussions during the course of the work.

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, and interatomic distances and angles for compound **2** (3 pages). Ordering information is given on any current masthead page.

Formal Total Synthesis of 1β-Methylcarbapenem via a Novel Route to Deoxyamino Sugars[†,‡]

Uko E. Udodong and Bert Fraser-Reid*

Department of Chemistry, Paul M. Gross Chemical Laboratory, Duke University, Durham, North Carolina 27706

Received June 27, 1988

Electrophilic amination of keto sugars provides an easy route to deoxyamino sugars. The usefulness of this procedure is demonstrated by the synthesis of lactone **5d**, a key intermediate for 1β-methylcarbapenem.

In carbohydrate transformations involving discrete electrophilic and nucleophilic partners, the sugar moiety usually functions in the former capacity.[1] However, nucleophilic displacements on sugar residues are comparatively difficult, owing to the inductive effect of the neighboring oxygen(s). This is exemplified in the preparation of deoxyamino sugars where the classical strategy involves displacement of a sulfonate ester with azide ion

[†]This work was supported by grants from the National Institutes of Health (Grant GM-32569) and Merck, Sharp, and Dohme (Rahway, NJ).

[‡]A preliminary account of this work has appeared: Udodong, U. E.; Fraser-Reid, B. *J. Org. Chem.* **1988**, *53*, 2131.

(1) (a) Ahmed, F. M. E. S.; David, S.; Vatele, J.-M. *Carbohydr. Res.* **1986**, *155*, 19. (b) Wolfrom, M. L.; Otani, S. *Carbohydr. Res.* **1986**, *153*, 150. (c) Rosenthal, A.; Catsoulacos, P. *Can. J. Chem.* **1969**, *47*, 2747 and references cited therein. (d) Richardson, A. C. *Carbohydr. Res.* **1967**, *4*, 422. (e) Ali, Y.; Richardson, A. C. *Carbohydr. Res.* **1967**, *5*, 441.

0022-3263/89/1954-2103$01.50/0