Mass Spectra of Germacranolide Type Sesquiterpene Dilactones and Derivatives

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The low and high resolution mass spectra of five germacranolide sesquiterpene dilactones together with the d_0-, d_3-acetates, d_0-, d_9-TMSi derivatives and deuterium exchange products were obtained. The mass spectra of the five dilactones revealed several kinds of common fragment ions. The germacranolides containing a 4,5 double bond exhibit characteristic ions at *m/e* 274, 256, 228, 165 and 147, while the 4,5-dihydro compound exhibits fragment ions at *m/e* 276, 258, 230 and 162.

INTRODUCTION

In spite of the rapidly growing interest in sesquiterpene lactones due to their various biological activities,[1,2] little attention has been directed toward the use of mass spectrometry in the structure elucidation of the different structural types of germacranolide-type sesquiterpene lactones.[3] In our directed phytochemical search for antitumor-active constituents within the tribe Heliantheae (Compositae) we have isolated over 20 lactonic constituents, in particular from the genus *Melampodium*.[4–6] Difficulties in the separation of closely related sesquiterpene lactones by the commonly used silica gel chromatography methods and ambiguities in the NMR spectral assignments of nonseparable mixtures has led to the application of GCMS analyses of the TMSi derivatives of hydroxyl-containing sesquiterpene lactone mixtures.[7] The use of GCMS for rapid separation and identification prompted a more detailed study of the mass spectral patterns of known compounds to be used as reference in future structure elucidations of other related plant constituents. This paper outlines typical mass spectral fragmentation patterns of five germacranolide dilactones—melampodin B (**1**), melampodin C (**3**), melampodin D (**4**), cinerenin (**5**) and 4,5-dihydromelampodin B (**2**).[8,9] It should be noted that most recent single crystal X-ray diffraction studies[9] on melampodin B demonstrated that, contrary to previous report,[8] this dilactone contains a 4,5-*cis* double bond and a 2-α-acetoxy group, as shown in **1**. On the further basis of highly similar NMR parameters, melampodin C (**3**) and D (**4**) appear to have the same configuration of the medium ring as **1**.

RESULTS AND DISCUSSION

The mass spectra of compounds **1–5** are presented in Table 1.

Melampodin B, C and D

The most typical fragmentation patterns of three closely related dilactones are outlined in Scheme 1. The mass spectrum of melampodin B (**1**), ($C_{17}H_{18}O_7$,

Table 1. Mass spectra of germacranolide dilactones (20 eV)

Compound	*m/e*/(relative intensity)																	
1	275 (20.0)	274 (97.6)	256 (*100*)	246 (40.8)	245 (48.0)	228 (76.2)	212 (11.0)	200 (39.0)	199 (34.2)	179 (27.2)	178 (35.8)	166 (12.5)	165 (59.5)	147 (37.5)	112 (29.0)	82 (16.0)	45 (9.8)	43 (11.6)
2	336 (62.9)	277 (10.4)	276 (33.7)	258 (60.0)	246 (40.0)	245 (9.2)	230 (*100*)	212 (35.7)	200 (26.5)	199 (15.3)	179 (11.9)	165 (27.5)	162 (45.7)	149 (17.0)	147 (17.9)	82 (13.0)	60 (10.1)	43 (65.7)
3	362 (0.55)	275 (9.2)	274 (44.0)	256 (41.5)	245 (12.8)	228 (32.2)	212 (4.0)	200 (13.5)	199 (14.2)	178 (9.5)	166 (5.8)	165 (30.0)	147 (18.5)	98 (17.5)	82 (12.0)	71 (*100*)	43 (87.0)	
4	376 (0.60)	275 (10.0)	274 (43.5)	256 (41.5)	246 (10.2)	245 (9.5)	228 (30.0)	212 (5.5)	200 (12.2)	199 (11.0)	178 (8.5)	166 (4.0)	165 (26.0)	147 (19.0)	85 (86.8)	82 (11.0)	74 (16.0)	57 (*100*)
5	320 (5.5)	275 (20.0)	274 (97.6)	256 (*100*)	246 (40.8)	245 (48.0)	228 (76.2)	212 (11.0)	200 (39.0)	199 (34.2)	179 (27.2)	178 (35.8)	166 (12.5)	165 (59.5)	147 (37.5)	112 (29.0)	82 (16.0)	45 (9.8)	43 (11.6)
5e	321 (7.0)	320 (2.7)	276 (18.5)	275 (*100*)	257 (26.5)	256 (69.0)	246 (34.0)	228 (54.0)	212 (11.0)	200 (24.0)	199 (23.0)	179 (50.5)	178 (38.0)	166 (43.5)	165 (39.5)	147 (35.0)	112 (47.5)	82 (17.0)	43 (11.0)

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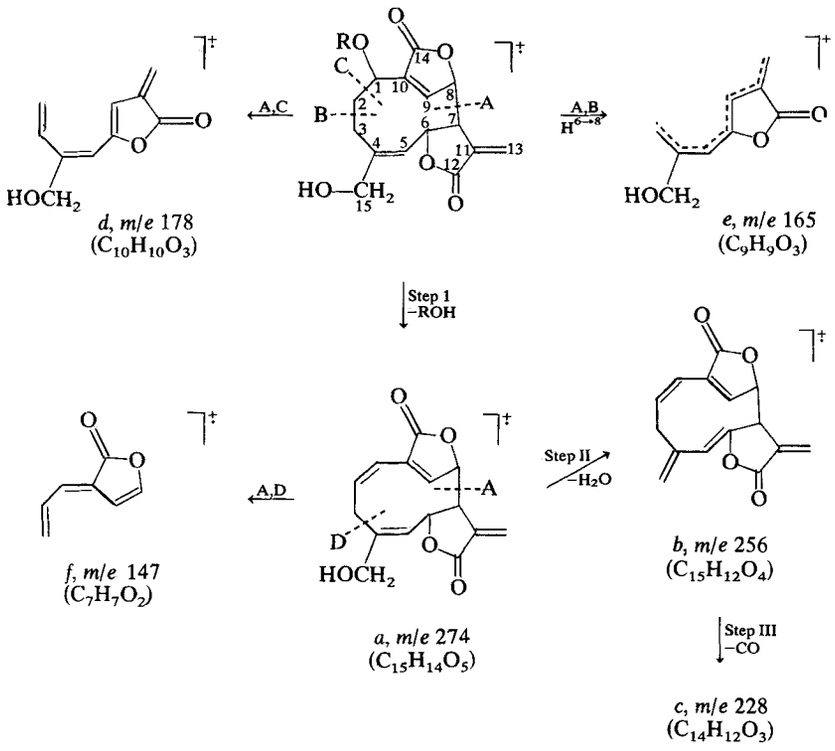


Scheme 1

mol. wt 334) does not give a molecular ion, but the C-1 acetyl function readily undergoes a McLafferty rearrangement with loss of ROH resulting in the characteristic fragment ion *a* at *m/e* 274.

Melampodin C (**3**), ($C_{19}H_{22}O_7$, mol. wt 362) and melampodin D (**4**), ($C_{20}H_{24}O_7$, mol. wt 376) both exhibit molecular ions. The major differences in their mass spectra result from their respective C-1 sidechains. In **3**, the ions at *m/e* 43 and 71 [Fig. 1(b)] are indicative of the isobutyrate moiety, while in **4** the ions at *m/e* 57 and 85 [Fig. 1(c)] are characteristic of the 2-methylbutanoate sidechain.

In **1**, **3** and **4**, loss of H_2O from ion *a*, thermally and/or by homolytic cleavage, provides ion *b* and extrusion of CO from ion *b*, either the C-12 or C-14 carbonyl group, forms ion *c*. Ions *a*, *b* and *c* comprise the major fragmentation pathway of the germacranolide dilactones **1**, **3**, **4** and **5** [see Fig. 1(*a–d*)]. Single crystal X-ray diffraction data of **1**[9] indicate considerable strain in the cyclodecadiene skeleton expressed in homolytic cleavage of the C-7,8 bond. Concomitant cleavage of the C-1,2, C-2,3 or C-3,4 bonds results in ions *d*, *e* and *f*.

The structures of the ions *d*, *e* and *f* are corroborated by the following observations: (1) deuterium exchange at the C-15 hydroxyl function of **1**, **3**, **4** and **5** increases the respective molecular ions and the ions *a*, *d* and *e* by 1 amu; (2) ions *b*, *c* and *f* remained unchanged in the monodeuterated compounds; (3) in the d_3-acetates **1b**, **3b**, **4b** and **5b** the fragment at *m/e* 166 and to a lesser extent the ion at *m/e* 179 are due to the elimination of $CD_2{=}C{=}O$ from the C-15 acetate function leaving a deuterium at the C-15 oxygen; (4) the d_0- and d_9-TMSi derivatives of **1**, **3**, **4**, **5** exhibit significant ions at *m/e* 237 and 246 (ion *e*+72, and ion *e*+81, respectively); (5) ion *f* remains unchanged with both labelled (C-15-OD) and unlabelled (C-15-OH) compounds. Similar fragmentation leading

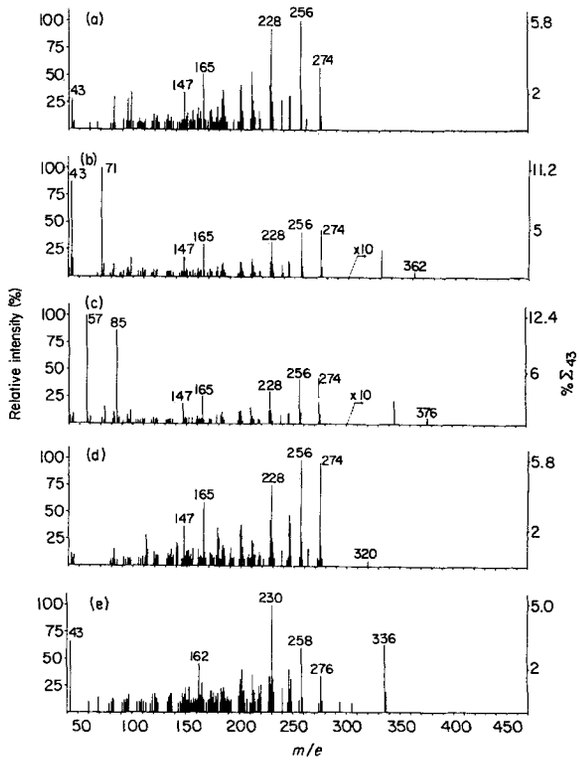


Figure 1. Mass spectra of germacranolide sesquiterpene dilactones: (a) melampodin B (**1**); (b) melampodin C (**3**); (c) melampodin D (**4**); (d) cinerenin (**5**); (e) 4,5-dihydromelampodin B (**2**).

to an analogous structure of ion *d* was reported for germacranolide dilactones.[3]

Cinerenin

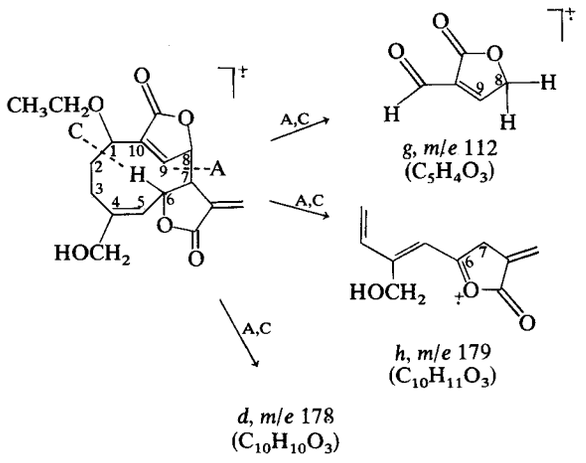
Cinerenin (**5**), ($C_{17}H_{20}O_6$, mol. wt 320) contains an ether function at C-1 which subtly influences its fragmentation pathway to differ from the C-1-acylated dilactones **1**, **3** and **4**. The C-1 ethoxy sidechain fragments to give the ions $[M-C_2H_5]^+$, $[M-C_2H_5O]^+$ and predominantly $[M-C_2H_5OH]^{+\cdot}$. After initial loss of C_2H_5OH, cinerenin follows the fragmentation pathway outlined in Scheme 1. Additionally, the 70 eV spectrum of **5** exhibits a base peak, ion *g* at *m/e* 112 ($C_5H_4O_3$). Scheme 2 rationalizes the formation of this ion by simultaneous homolytic cleavage of the strained C-1,2 and C-7,8 bonds and loss of $C_2H_5\cdot$ from the C-1 oxygen. The required hydrogen migration to form ion *g* very likely involves hydrogen transfer from C-6 to C-8, which is sterically most favored as gleaned from X-ray crystallographic data.[9]

Cinerenin also exhibits major ions *h* at *m/e* 179 and *d* due to A,C-cleavages. Ion *h* may stabilize by loss of hydrogen forming the more intense ion *d*. Ions *d*, *g* and *h*, which are more intense in cinerenin than in the C-1 acylated compounds **1**, **3** and **4**, can be used as diagnostic markers for the presence of a C-1 ether linkage.

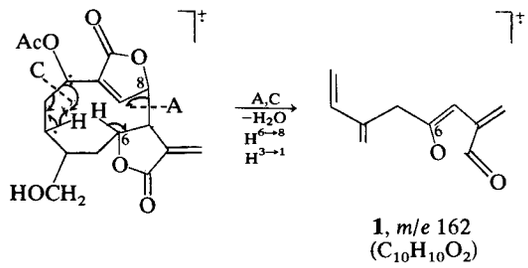
4,5-Dihydromelampodin B

4,5-Dihydromelampodin B (**2**), ($C_{17}H_{20}O_7$, mol. wt 336) contains a saturated C-4,5 bond; consequently, its medium ring portion is less strained than those of **1**, **3**, **4** and **5**. Due to this flexibility, **2** exhibits an intense molecular ion in contrast to the strained germacranolide dilactones [Fig. 1(e)]. The mass spectrum of **2** differs from those of the other dilactones in that the characteristic ions *a*, *b* and *c* in compounds **1**, **3**, **4** and **5** appear two amu higher at *m/e* 276, 258 and 230 in **2**, providing further support for steps I–III in Scheme 1. The intense ion *i* at *m/e* 162 is postulated to be formed via homolytic cleavages of the C-1,2 and C-7,8 bonds with simultaneous loss of H_2O via β-cleavage of H-4 (Scheme 3). The two noted hydrogen migrations are sterically favorable.

The fragmentation patterns of the d_3-acetate (**2b**) and the deuterium exchange product (**2c**) substantiate the fragmentation outlined in Scheme 3.



Scheme 2



Scheme 3

Table 2. Mass spectra of germacranolide dilactones: (a) d_0- and (b) d_3-acetates (20 eV)

Compound	m/e/(relative intensity)																		
1a	376	332	317	316	275	274	273	256	228	212	179	178	166	165	147	98	82	60	43
	(8.0)	(18.5)	(34.5)	(11.2)	(22.0)	(*100*)	(46.5)	(95.5)	(89.9)	(55.5)	(6.8)	(26.8)	(9.0)	(40.0)	(28.8)	(27.0)	(33.8)	(8.5)	(56.5)
1b	382	379	338	276	275	274	273	256	228	212	178	166	165	147	82	63	46	43	
	(6.0)	(4.0)	(12.0)	(15.6)	(44.4)	(55.0)	(17.6)	(60.0)	(62.2)	(48.0)	(20.2)	(36.0)	(10.0)	(21.8)	(27.2)	(44.4)		(12.0)	
2a	378	336	319	318	277	276	275	258	230	212	162	149	60	43					
	(*100*)	(23.0)	(5.8)	(12.2)	(16.0)	(81.0)	(7.5)	(91.8)	(71.5)	(40.5)	(44.0)	(23.2)	(6.0)	(50.0)					
2b	384	381	340	321	278	277	276	275	258	230	212	166	165	162	149	147	63	46	43
	(87.6)	(42.0)	(22.0)	(14.0)	(15.0)	(67.6)	(42.4)	(16.2)	(*100*)	(76.0)	(44.6)	(20.0)	(24.4)	(53.0)	(35.6)	(12.0)	(21.6)	(80.0)	(15.8)
3a	404	360	317	275	274	273	256	228	212	178	166	165	147	82	71	60	43		
	(3.0)	(5.4)	(29.8)	(9.6)	(27.6)	(38.0)	(29.6)	(38.2)	(28.2)	(7.6)	(5.6)	(12.0)	(9.0)	(10.0)	(45.0)	(18.6)	(*100*)		
3b	407	363	320	277	276	275	274	256	228	212	178	166	165	147	82	71	63	46	43
	(4.0)	(12.0)	(63.0)	(23.0)	(*100*)	(44.0)	(17.8)	(73.0)	(74.0)	(72.0)	(15.0)	(31.0)	(10.0)	(19.0)	(18.6)	(*100*)	(13.0)	(73.6)	(82.0)
4a	418	374	317	275	274	273	256	228	212	178	166	165	147	98	85	82	74	60	57	43
	(6.0)	(7.8)	(67.0)	(27.6)	(56.0)	(86.0)	(68.0)	(65.0)	(62.0)	(20.0)	(14.0)	(26.2)	(16.0)	(15.0)	(*100*)	(15.8)	(82.0)	(25.6)	(95.0)	(88.0)
4b	421	377	320	276	275	274	256	228	212	178	166	165	147	85	82	74	63	57	46	45
	(5.0)	(5.0)	(34.0)	(50.0)	(23.0)	(7.2)	(41.0)	(31.0)	(39.6)	(10.0)	(12.0)	(6.0)	(7.0)	(46.0)	(12.4)	(29.0)	(26.4)	(44.0)	(*100*)	(35.0)
5a	362	333	318	317	316	302	275	274	273	256	228	212	179	178	166	165	147	112	82	43
	(1.9)	(0.7)	(8.0)	(6.8)	(13.6)	(5.3)	(17.2)	(99.1)	(7.8)	(*100*)	(54.3)	(12.8)	(16.5)	(18.5)	(5.0)	(27.8)	(16.5)	(16.6)	(7.5)	(16.0)
5b	365	321	320	319	302	277	276	275	274	273	256	228	212	179	178	166	165	147	112	46
	(7.5)	(12.6)	(12.0)	(26.0)	(5.5)	(15.8)	(58.8)	(26.0)	(*100*)	(22.0)	(63.5)	(95.6)	(29.0)	(38.0)	(27.0)	(34.0)	(19.0)	(27.2)	(41.5)	(45.0)

Table 3. Mass spectra of germacranolide dilactones: (c) d_0- and (d) d_9-TMSi derivatives (20 eV)

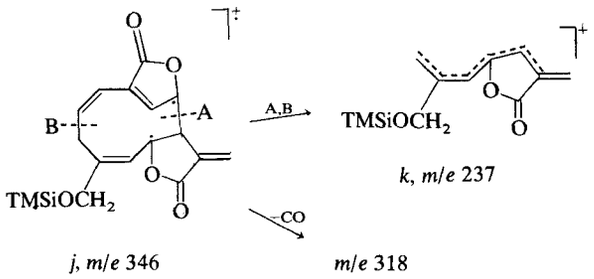
Compound	$[M]^+$	$[M-CH_3]^+$ $[M-CD_3]^+$	$[M-OR]^+$	$[M-ROH]^+$	$[M-ROH-CO]^+$	Other						
1c	406	391	347	346	318	256	237	228	73	43		
	(0.6)	(25.5)	(34.0)	(*100*)	(93.5)	(14.5)	(14.0)	(12.8)	(31.0)	(4.5)		
2c	408	493	349	348	320				73	43		
	(3.6)	(*100*)	—	—	(32.0)				(18.0)	(6.0)		
3c	434	419	397	346	318	256	237	228	147	73	71	43
	(4.4)	(6.0)	(53.0)	(55.0)	(65.0)	(44.0)	(19.0)	(25.6)	(34.0)	(50.0)	(*100*)	(55.0)
4c	448	433	347	346	318	256	237	228	147	85	73	57
	(2.6)	(4.6)	(42.0)	(47.6)	(60.0)	(46.0)	(16.8)	(25.0)	(26.0)	(*100*)	(43.2)	(98.4)
5c	392	377	347	346	318	256	237	228	147	73		
	(15.0)	(4.1)	(20.0)	(54.0)	(*100*)	(19.5)	(11.2)	(18.5)	(34.5)	(72.5)		
1d	415	397	356	355	327	256	246	228	147	82	43	
	(11.6)	(9.6)	(74.0)	(*100*)	(60.0)	(57.0)	(26.0)	(27.0)	(24.2)	(50.0)	(13.0)	
2d	417	399	358	357	329	162		82	43			
	(3.0)	(*100*)	—	—	(37.0)	(9.0)		(21.2)	(6.5)			
3d	443	425	356	355	327	256	246	228	147	82	71	43
	(9.0)	(6.8)	(66.0)	(*100*)	(65.0)	(60.0)	(20.0)	(26.0)	(22.0)	(36.2)	(80.0)	(34.0)
4d	457	439	356	355	327	256	246	228	147	85	82	57
	(6.2)	(5.5)	(56.5)	(*100*)	(67.5)	(64.5)	(19.0)	(23.5)	(25.0)	(79.0)	(38.5)	(64.0)
5d	401	383	356	355	327	256	246	228	147	82		
	(29.5)	(3.3)	(34.0)	(*100*)	(94.1)	(17.2)	(14.8)	(11.5)	(19.6)	(32.5)		

d_0- and d_3-Acetate derivatives

The mass spectra of the d_0- and d_3-acetate derivatives of **1**, **2**, **3**, **4** and **5** are listed in Table 2. The acetate derivatives **1b** and **2b** exhibit more complex spectra due to transesterification of the C-1 acetate sidechain with a d_3-acetyl group during the d_6-acetic anhydride treatment of **1** and **2**. Thus, instead of peaks due to the mono C-15 d_3-acetate of **1** and **2**, the mass spectra contained peaks due to the equilibrium mixture of the d_3-mono and diacetate of **1** and **2**, with the peaks due to the C-1 and C-15 d_3-diacetates predominating in the spectra.

d_0- and d_9-TMSi derivatives

The mass spectra of the d_0- and d_9-TMSi derivatives of **1–5** are summarized in Table 3. Intense fragment ions are observed at *m/e* 346 (*j*), 318 and 237 (*k*) (Scheme 4) and the corresponding fragments of the d_9-TMSi derivative are found at *m/e* 355, 327 and 246. The mass spectrum of **3c** [Fig. 2(*b*)] is typical of the TMSi derivatives.



j, *m/e* 346 *k*, *m/e* 237 *m/e* 318

Scheme 4

CONCLUSIONS

The mass spectra of cinerenin, melampodins B, C and D exhibit characteristic ions *a*, *b*, *c*, *d*, *e* and *f*, whereas

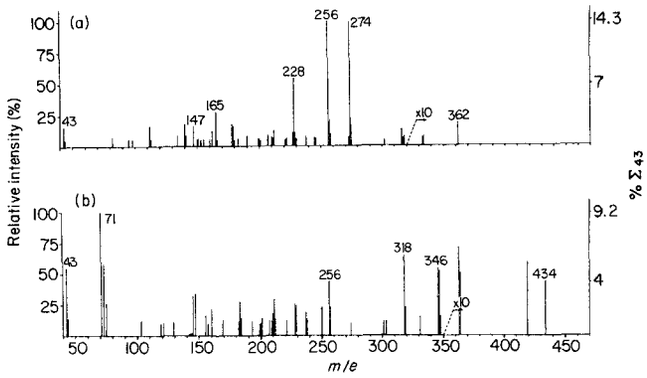


Figure 2. Mass spectra of germacranolide sesquiterpene dilactone derivatives: (a) cinerenin acetate (**5a**); (b) melampodin C TMS (**3c**).

4,5-dihydromelampodin B contains ions at *m/e* 276, 258, 230 and 162. The respective d_0-, d_3-acetates, d_0-, d_9-TMSi derivatives, deuterium exchange products and high resolution mass spectra corroborate the general pathway represented in Scheme 1.

Ring strain due to the 4,5 and 9,10 double bonds in melampodin B, melampodin C, melampodin D and cinerenin dictate the type of fragments resulting in ions *d*, *e* and *f*, while the more flexible medium ring in 4,5-dihydromelampodin B permits formation of a different fragment, ion *i*. The difference between the oxygen bond of the C-1 ether linkage in cinerenin and the C-1 ester linkage in the remaining dilactones explains the difference in ion intensities of ions *d*, *g* and *h* in cinerenin. Similarly, the C-15 acetate derivatives of these germacranolide sesquiterpene dilactones fragment quite readily with loss of $CH_2CO(CD_2CO)$, $CH_3CO(CD_3CO)$ and $CH_3COOH(CD_3COOH)$, while the C-15 silyl ether linkage is relatively stable to fragmentation of the C-15 O—Si bond.

EXPERIMENTAL

Low resolution mass spectra were obtained on a LKB-9000 interfaced with a 9 ft × 4 mm i.d. silanized glass column packed with 1% SE-30 on Gas Chrom Q (100/200 mesh). The instrumental conditions for the LKB-9000 were (direct introduction probe or GCMS): ionizing voltage 20 eV, ionizing current 60 μA; ion source temperature 270 °C; accelerating voltage −3.5 kV. High resolution mass spectra were obtained on a DuPont/CEC 21-110B under the following conditions: ionizing voltage 70 eV; ionizing current 100 μA; accelerating voltage −8.4 kV; ion source temperature 280 °C. An Ilford Q2 photoplate served as the ion detector. Data from the photoplate were obtained by means of a microdensitometer-comparator-computer system described previously.[10] Reported elemental compositions agree to within three millimass units of their respective theoretical values.

The d_0- and d_3-acetate derivatives were prepared using acetic anhydride (d_0- and d_6-) in pyridine for 3 h at 60 °C. The acetate derivatives were extracted with $CHCl_3$ after neutralization with saturated $NaHCO_3$ and prepared for the direct inlet probe. The TMSi derivatives were made using BSTFA (d_0-) and d_{18}-BSA and heating for 3 h at 60 °C. The TMSi derivatives were injected without further purification into the GCMS system and temperature programmed from 200–260 °C.

Acknowledgements

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