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GAS CHROMATOGRAPHY/MASS SPECTROSCOPY OF ISOFLAVANONES AND RELATED COMPOUNDS

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Abstract—Trimethylsilylation of five isoflavanones led to the formation of two products (in the case of kievitone) or three products (for all other isoflavanones). These products were separated by GC and their electron impact mass spectra were obtained. In all but one case, the last component to elute from the column was the pertrimethylsilyl ether of the isoflavanone. The other products appeared to be isomeric with the isoflavanones. These products were tentatively identified as the pertrimethylsilyl derivative of an enol and the pertrimethylsilyl ether of a ring-opened compound isomeric with chalcones.

INTRODUCTION

Isoflavanones have been reported to be intermediates in the biosynthesis of antifungal pterocarpans and isoflavans[1] and some isoflavanones have antifungal activity[2, 3]. For example, kievitone [5,7,2',4'-tetrahydroxy-8-(3'',3''-dimethylallyl)-isoflavanone] is one of the principal isoflavonoid phytoalexins produced by *Phaseolus vulgaris*[4]. *P. vulgaris* has been shown to produce smaller amounts of dalbergioidin (5, 7, 2', 4'-tetrahydroxyisoflavanone), 7, 2', 4'-trihydroxyisoflavanone and 5-deoxykievitone also as a result of fungal infection[5–7]. The former two appear to be precursors of kievitone and phaseollin (the major pterocarpan formed as a result of fungal infection), respectively, while 5-deoxykievitone appears to be a branch from the phaseollin pathway[7]. Previous attempts to estimate kievitone by GC using hexamethyldisilizane-trimethylchlorosilane as the silylating reagent were not successful[8]. This failure may have been due to incomplete silylation[9]. Recently, several isoflavonoids including kievitone and other isoflavanones were detected in extracts of *P. vulgaris* and *Trifolium repens* by GC/MS of trimethylsilyl (TMSi) ethers prepared using bis-trimethylsilyl-trifluoroacetamide as the silylating reagent[10, 11]. Kievitone gave two products and all of the other isoflavanones gave three products. In each case, one product had a molecular ion at the expected mass of the isoflavanone TMSi ether and two products (one in the case of kievitone) had molecular ions 72 mass units above the mass of the isoflavanone TMSi ether. It was also observed that the proportions of the products varied with the period of time between silylation and injection and from sample to sample. These observations strongly indicated that the additional products were due to the formation of isomers rather than due to decomposition.

Although no other studies have dealt with GC or GC/MS of isoflavanones, several reports have appeared in which the analogous flavanones have been studied. Trimethylsilylation of flavanones produces one to three products depending on reagents and reaction conditions[9, 12–14]; however, the nature of the products has not been established. Methylation of the flavanone naringenin under strongly basic conditions resulted in ring opening to give the permethylated ether of the corresponding chalcone[15]. The conditions required for trimethylsilylation of flavanones are mild, but formation of a chalcone during the reaction is feasible. A similar reaction is possible in the case of isoflavanones. Isoflavanones appear to be formed from isoflavones by the stereospecific reduction of the C-2–C-3 double bond[16], yet most naturally occurring isoflavanones are optically inactive. This is probably the result of enolization of the carbonyl group[17]. Although formation of enolic TMSi ethers of flavonoids has not been reported, silylation of an analogous compound, 4-hydroxy-3-methoxyacetophenone, gave both a mono-TMSi and an enolic di-TMSi ether under certain conditions[18]. The identity of the products was confirmed by GC/MS and IR[18].

RESULTS AND DISCUSSION

Trimethylsilylation of most isoflavanones gave three products; however, only two products were observed in the case of kievitone. The products will be designated I, II and III in order of elution from the GC column. The products of kievitone will be designated I and III. Silylation product III had a molecular ion at the calculated m/z for the isoflavanone TMSi ether in all but one case. The exception was 7,4'-dihydroxyisoflavanone (dihydrodaidzein) in which silylation product II was found to be the isoflavanone TMSi ether. Abbreviated mass spectra of the TMSi ethers of dihydrodaidzein (**1a**), 7, 2'-dihydroxy-4'-methoxyisoflavanone (vestitone) (**2a**),

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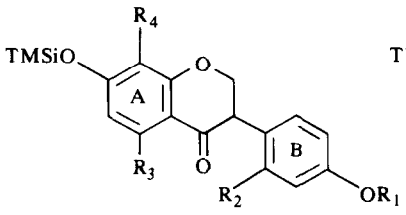
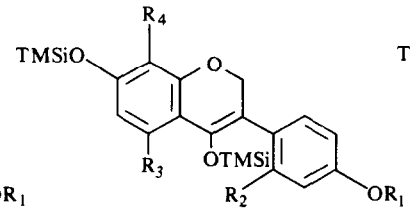
Table 1. Retention times and major ions in the mass spectra of the trimethylsilylation products of isoflavanones

Proposed structure	Retention time (min)	Major ions [m/z (%)]															
1a	4.9	400	(6),	385	(8),	281	(31),	192	(100),	177	(27),	73	(17)				
1b	5.6	472	(100),	471	(81),	457	(32),	383	(16),	281	(49),	191	(6),	73	(79)		
1c	3.9	472	(23),	471	(5),	457	(25),	383	(2),	281	(100),	191	(4),	73	(51)		
2a	5.9	430	(16),	415	(6),	281	(11),	222	(100),	207	(30),	73	(20)				
2b	4.7	502	(100),	501	(54),	487	(52),	413	(19),	281	(46),	221	(5),	73	(64)		
2c	3.9	502	(50),	501	(5),	487	(29),	413	(3),	281	(100),	221	(8),	73	(40)		
3a	7.2	488	(10),	473	(5),	281	(26),	280	(100),	265	(16),	73	(77)				
3b	6.0	560	(100),	559	(35),	545	(52),	471	(21),	281	(78),	279	(17),	73	(95)		
3c	4.4	560	(77),	559	(8),	545	(42),	471	(5),	281	(100),	279	(17),	73	(54)		
4a	8.6	576	(3),	561	(15),	369	(46),	280	(100),	265	(21),	73	(65)				
4b	6.8	648	(77),	647	(12),	633	(21),	559	(22),	369	(70),	279	(4),	73	(100)		
4c	5.3	648	(46),	647	(2),	633	(11),	559	(3),	369	(63),	279	(3),	73	(100)		
5a	18.0	644	(7),	629	(70),	437	(7),	364	(100),	349	(47),	280	(49),	265	(25),	73	(97)
5b	N.D.																
5c	9.2	716	(96),	715	(11),	701	(47),	627	(10),	437	(100),	279	(6),	73	(59)		

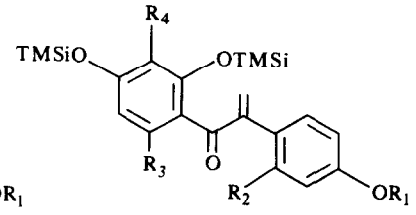
N.D. = not detected.



(a)

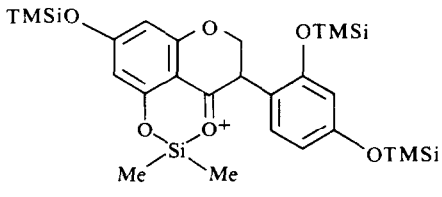


(b)



(c)

	R_1	R_2	R_3	R_4
1	TMSi	H	H	H
2	Me	OTMSi	H	H
3	TMSi	OTMSi	H	H
4	TMSi	OTMSi	OTMSi	H
5	TMSi	OTMSi	OTMSi	$CH_2CH=CMe_2$



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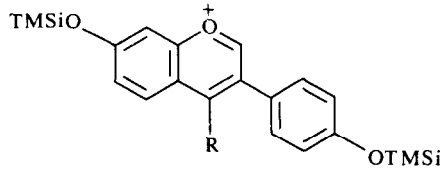
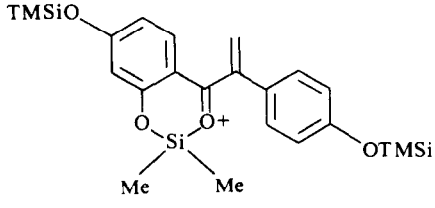


7 R = OTMSi

8 R = H



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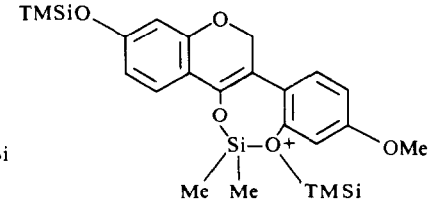

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7, 2', 4'-trihydroxyisoflavanone (**3a**), dalbergioidin (**4a**) and kievitone (**5a**) are given in Table 1 together with their retention times. The mass spectra of the isoflavanone TMSi ethers **1a–4a** all showed the base peak as the retro Diels–Alder (RDA) fragment ion with charge retention on ring B. All compounds showed a medium to low intensity ion corresponding to a RDA fragmentation with silyl transfer and charge retention on ring A. All of the compounds examined showed weak molecular ions; the TMSi ethers of dalbergioidin (**4a**) and kievitone (**5a**) showed relatively much more abundant $[M-15]^+$ ions. This feature is characteristic of 5-OTMSi substituted isoflavones[19]. For the TMSi ether of dalbergioidin (**4a**), the $[M-15]^+$ ion probably results from the formation of the resonance stabilized ion **6**. The major fragment ions in the mass spectrum of the TMSi ether of kievitone appear to arise from the same cleavages as in underivatized kievitone[20], except that the RDA fragment corresponding to ring

A occurs with hydrogen transfer in the spectrum of kievitone but without hydrogen transfer in the spectrum of **5a**.

Structure **b** is an enol ether of an isoflav-3-ene. The mass spectrum of the permethyl ether of the isoflav-3-ene sepiol shows a major $[M-1]^+$ ion[21], which is due to the facile formation of an isoflavylium ion[22]. The mass spectra of TMSi ethers of compounds with structure **b** might be expected to show prominent $[M-1]^+$ and $[M-89]^+$ ions. Isoflavylium ions **7** and **8** could be obtained from the enol TMSi ether of dihydrodaidzein (**1b**) by the loss of hydrogen and OTMSi (with hydrogen migration), respectively. Inspection of structure **c** shows that similar resonance stabilized ions cannot be formed readily. The mass spectra of silylation product III of dihydrodaidzein and silylation product II of vestitone and 7, 2', 4'-trihydroxyisoflavanone show major $[M-1]^+$ ions and medium intensity $[M-89]^+$ ions whereas the corresponding ions are relatively much smaller in the spectrum of silylation product I of these compounds (Table 1). Based on the above data, it is proposed that silylation product III of dihydrodaidzein has structure **1b** and that silylation product II of all of the other isoflavanones is the enol TMSi derivative. The products which have been tentatively identified as enolic ethers based on the above mass spectral evidence were almost always the least abundant of the products. This product represented less than 5% of the total of the three products in the case of dalbergioidin and this product was not obtained from kievitone. These observations are also consistent with the proposed assignments as the formation of the enolic per-TMSi ethers of dalbergioidin (**4b**) and kievitone (**5b**) would not be expected to proceed readily due to steric hindrance.

It is proposed that product I of each isoflavanone has structure **c**. This is based primarily on differences in the mass spectrum and retention times of the products from dihydrodaidzein. A difference is observed in the intensity of the $[M-15]^+$ ion relative to the molecular ion for the silylation products I and III of dihydrodaidzein. Product I has the $[M-15]^+$ ion of equal intensity to the molecular ion while product III has the corresponding ion only one-third the intensity of the molecular ion (Table 1). If product I has structure **c**, the ion formed (**9**) would be resonance stabilized. A similar ion cannot be obtained from the enol isomer. The difference in relative intensities of the $[M-15]^+$ ions in the spectra of the corresponding products of the other isoflavanones is not apparent. This is probably due to the presence of the 2'-OTMSi group which can undergo ring formation by loss of a methyl group from either the 2'-OTMSi group or the enolic OTMSi group. The resulting ion (i.e. **10** in the case of **2b**) would have a strong tendency to lose an intact TMSi group[23]. The charge will be retained on the TMSi group in the second step of the reaction[23]. Compound **1c** is structurally very different from **1b**. Compound **1c** has free rotation around the carbonyl while **1b** is rigid. This difference might be expected to lead to widely different retention times for **1b** and **1c** with the less rigid **1c** eluting prior to **1b** on this apolar column. The data in Table 1 are consistent with product I having structure **1c** (and similarly for the other isoflavanones).

The presence of three silylation products of the chalcone isoliquiritigenin has also been observed[10]. The mass spectra show that there are two products with an $M^{+\cdot}$ at m/z 472 and one product with an $M^{+\cdot}$ at m/z 400. This indicates that there are two tri-TMSi derivatives (probably the chalcone and a flav-3-en-4-ol) and a single di-TMSi ether (probably the flavanone). The mass spectra of the two tri-TMSi derivatives were nearly identical, hence structural assignments cannot be made on the basis of the mass spectra.

The results obtained in this and other studies indicate that two or three silylation products for isoflavanones, flavanones and probably for 2'-hydroxylated chalcones are obtained under some silylation conditions. One of the most powerful tools for the identification of many types of flavonoids is ^{1}H NMR of the TMSi ethers [24]. It thus appears that GC/MS of isoflavanone (and flavanone and 2'-hydroxychalcone) silylation products prior to ^{1}H NMR studies would help to determine whether or not a mixture is present, what the proportions of the products are, and the probable identity of the products, based on the mass spectral fragmentation patterns. The formation of three silyation products of isoflavanones, while unsatisfactory for quantitative analysis, could be very useful for the identification of naturally occurring isoflavanones especially as there are many possible isomers. The usefulness of GC/MS for the identification of the TMSi ethers of isomeric isoflavones has been demonstrated[19]. Both retention indices and fragmentation patterns were found to be valuable for differentiating the isoflavones. Derivatization of most isoflavanones should provide a unique set of retention indices and the fragmentation pattern should provide evidence for the positions of substituents[19].

EXPERIMENTAL

TMSi derivatives were prepared by mixing pyridine–BSTFA (1 : 1) with the sample and heating to 60° for 5 min. The derivatives were chromatographed on a glass column (1.5 × 2 mm) packed with OV-101 (1.5%) on Gas-Chrom Q (80–100 mesh). The He carrier gas flow rate was 24 ml/min, the column was maintained at 215°, the injector temp. was 230° and the jet separator and lines to the mass spectrometer were maintained at 240°. Electron impact mass spectra were obtained at an ionization voltage of 70 eV. The accelerating voltage was 4 kV, the ion source temp. was 210° and the emission current was 200 μA.

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