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Linked Scan Measurements for the Localization of Side-chain Double Bonds in 9,19-Cyclopropano Steroids

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9,19-Cyclopropano steroids do not show the typical fragmentation reactions of unsaturated C-17 side-chains in their EI spectra. They can, however, be observed in linked scan measurements of the main fragment arising from ring B cleavage. © **1997 John Wiley & Sons, Ltd.**

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INTRODUCTION

Sterols and tetracyclic triterpenoids with a 9,19-cyclopropane ring can be recognized readily from their typical fragmentation behavior.[1,2] Side-chain unsaturation, which for many steroid results in highly characteristic and pronounced cleavage reactions,[3] seems to have only a minor influence or no influence at all on the breakdown pattern. In the course of the structure elucidation[4] of three terpenoids from *Skimmia wallichii* **1**–**3** we could show that this problem can be circumvented by linked scan measurements.

EXPERIMENTAL

70 eV mass spectra of **1**–**3** obtained with an HSQ-30 instrument (Finnigan-MAT, Bremen), *m/z* (%) (relative intensity):

Skimmiwallinin (**1**): 482 ($M^{\cdot+}$, 25), 467 (**a**, 16), 450 (**b**, 100), 435 (**c**, 47), 407 (**d**, 42) 381 (**e**, 17), 329 (**g**, 10), 328 (**h**, 25), 297 (**i**, 13), 230 (**p**, 13), 203 (**k**, 33), 201 (**l**, 21), 175 (**m**, 46), 173 (**n**, 25).

Skimmiwallichin (**2**): 496 ($M^{\cdot+}$, 21), 481 (**a**, 13), 464 (**b**, 100), 449 (**c**, 33), 421 (**d**, 29), 395 (**e**, 17), 342 (**h**, 21), 297 (**i**, 10), 230 (**p**, 17), 203 (**k**, 23), 201 (**l**, 13), 175 (**m**, 33), 173 (**n**, 15).

Skimmiwallin (**3**): 482 ($M^{\cdot+}$, 17), 467 (**a**, 22), 450 (**b**, 100), 435 (**c**, 37), 407 (**d**, 33), 381 (**e**, 22), 328 (**h**, 17), 297 (**i**, 15), 216 (**o**, 39), 203 (**k**, 48), 201 (**l**, 30), 175 (**m**, 48), 173 (**n**, 30).

For linked scan (B/E = const.) measurements the gas pressure in the collision cell was adjusted to reduce the intensity of the parent ion to 50% of its original value.

RESULTS AND DISCUSSION

According to Audier *et al.*[1] and Aplin and Hornby,[2] tetracyclic triterpenoids and steroids with a 9,19-cyclopropane ring undergo diagnostically significant cleavage reactions (for further examples see, Ref. 5). The most important one involves fission of the 9,10-, 9,19- and 5,6-bonds in $M^{\cdot+}$ with rearrangement of one H, resulting in ion **h** (Scheme 1), which may subsequently lose the entire side-chain (**m**; incidentally, parent ion scans of **m** indicate that this ion can be formed from almost any precursor ion and not—as had been assumed[2]—only from **h**). The mass of the ion **h** is shifted in accordance with the substituents of the side-chain. As parent ion scans show, it originates not only from $M^{\cdot+}$ but also from $[M - CH_3]^+$.

Two more fragments seem to be characteristic, namely (in the case of a 3-CH_3O group) $[M - CH_3OH - C_3H_7]^+$—possibly **d** as depicted in Scheme 1—and[1] $[M - CH_3OH - C_5H_9]^+$ (**e** in Scheme 1). For other ions—mainly combination losses—see Scheme 1.

9,19-Cycloterpenoids frequently have an unsaturated side-chain. For compounds without this cyclopropane ring, side-chain double bonds usually give rise to characteristic and abundant fragments.[3] In particular, those with a 24-methylene (as in **3**) or 24-ethylidene group (24,

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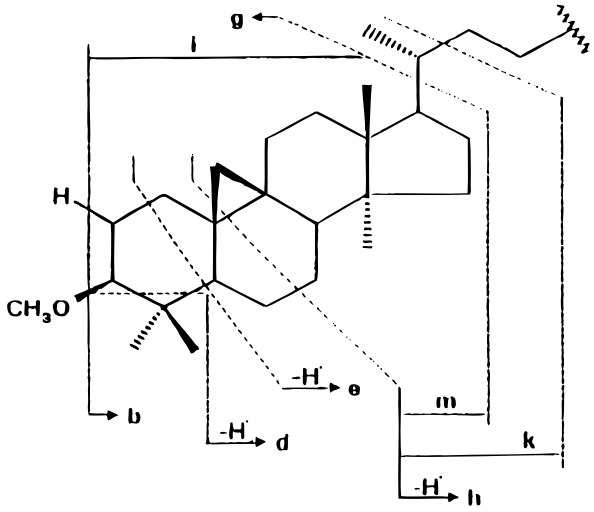
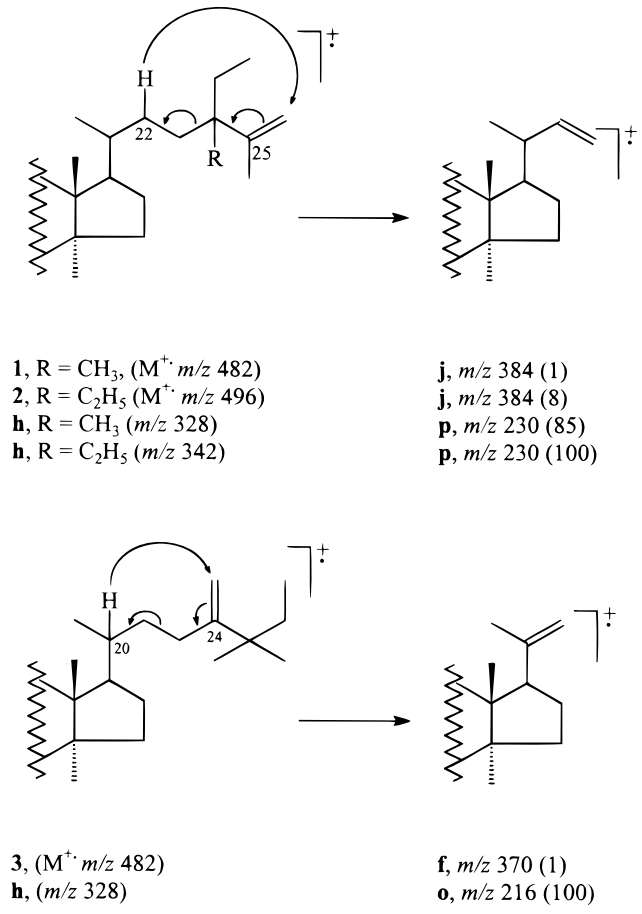


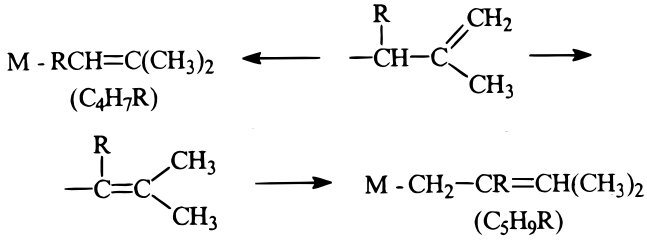
Scheme 1. Common fragments of **1–3** (for side-chains see Scheme 2): **a** $[M-CH_3]^+$, **b** $[M-CH_3OH]^{\cdot+}$, **c** $[M-CH_3OH-CH_3]^+$, **d** $[M-CH_3OH-C_3H_7]^+$, **e** $[M-CH_3OH-C_5H_9]^+$, **g** [M − side-chain]$^+$, **h** $[M-154]^{\cdot+}$, **i** [**b** − side-chain]$^+$, **k** [M − C-22 to C-27]$^+$, **l** [**k** − 2H]$^+$, **m** [**h** − side-chain]$^+$, **n** [**m** − 2H]$^+$.

24^1-unsaturation) undergo McLafferty rearrangement with transfer of the C-20 H (see Scheme 2, compound **3**). In the case of **3** this process would result in the loss of C_8H_{16} (M − 112; **f**, *m/z* 370), absent in the EI spectrum. Even a daughter ion (*B/E* = const.) scan for $M^{\cdot+}$, which should enhance the relative abundance of McLafferty rearrangements ions, yields *m/z* 370 only with a relative intensity of 1%.

The side-chain fragmentation induced by a 25,26-double bond is somewhat problematic as far as the literature is concerned. Wyllie and Djerassi[3] (and based on their work a review[6]) state that Δ^{25}-compounds also undergo McLafferty rearrangement (see Scheme 2, compounds **1** and **2**), quoting a paper by Bergmann *et al.*,[7] which, however, deals only with $24,24^1$-unsaturation (see above). Vulfson and Zaikin[8] in their review article mention a 24-ethyl-Δ^{25}-steroid which loses not—as expected—C_6H_{12}, but rather C_7H_{14}, and they explain this by the consecutive loss of $C_6H_{11}^{\cdot}$ (allylic cleavage) and $CH_3^{\cdot}$. Further examples of the loss of $C_{n+1}H_{2n+2}$ instead of C_nH_{2n} from Δ^{25}-steroids can be found in the literature (see e.g. Refs 9–11). A more likely explanation would be a shift of the double bond into the tetrasubstituted 24,25-position with subsequent McLafferty rearrangement. 24-Methyl-Δ^{24}-steroids show this rearrangement ion with high intensity,[7,12] while in the spectra of Δ^{24}-steroids lacking a 24-substituent, where the double bond is not tetrasubstituted, it is missing[7] (see also Refs 13 and 14). An indication of this double-bond migration is the observation that for the sterically hindered 24-isopropyl-Δ^{25}-cholesterol both $[M-C_7H_{14}]^{\cdot+}$ (*m/z* 328, 17%) and $[M-C_8H_{16}]^{\cdot+}$ (*m/z* 314, 28%) are present.[15]



Scheme 2. Side-chain fragmentation by McLafferty rearrangement (relative intensities in parentheses).



This double-bond migration should not be possible for 24,24-disubstituted Δ^{25}-steroids, but for the only

Table 1. Daughter ion (*B/E* = const.) linked scan spectra for h [masses and (in parentheses) relative abundances]

Compound	Parent ion *m/z*	h − 15	o	p	l	m	n	Other ions
1	328	313	—	230	201	175	173	285, 257
		(32)		(85)	(63)	(85)	(100)	(18), (2)
2	342	327	—	230	201	175	173	285, 257
		(20)		(100)	(13)	(11)	(13)	(3), (4)
3	328	313	216	—	201	175	173	285, 257
		(10)	(100)		(15)	(15)	(26)	(1), (1)

examples without a 9,19-ring known to us where partial mass spectra are reported,[5] side-chain fragments are not mentioned. In any case, for **1** and **2** in the EI spectra, ions **j** (or their lower homologs) are not observed. In the daughter ion (B/E = const.) linked scans of $M^{\cdot+}$ they are of low abundance (1% and 8% respectively).

It is therefore important to note that the daughter ion spectra (linked scan B/E = const.) of **h** (Table 1) showed the expected McLafferty fragments (Scheme 2) in high abundance (**1**, **2**: **p**, m/z 230; **3**: **o**, m/z 216). In addition, **m** (m/z 175, loss of the entire side-chain), **n** (m/z 173, **m** − 2H) and **l** (loss of C-22 to C-27 + 2H) originating from **h** can be seen. These results show that the B/E linked scan spectra can readily be used for locating sites of unsaturation in the side-chain where the EI spectra do not give the expected results.

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