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Fast Atom Bombardment Mass Spectrometry for the Characterization of Taxanes†

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Fast atom bombardment mass spectrometry (FABMS) of several taxanes and 11(15 → 1)*abeo*-taxanes having a 4(20)-exocyclic methylene group or oxetane ring was studied in detail using collision-induced dissociation (CID) of their $[M + Li]^+$, $[M + Na]^+$ and $[M + NH_4]^+$ ions. The results indicate that FAB followed by MS/MS is a valuable tool for the identification of functional groups in taxanes and to some extent the type of taxane under investigation. The presence of hydroxy groups in the molecule is best confirmed by examining the CID of the $[M + NH_4]^+$ adduct ions. The 11(15 → 1)*abeo*-taxanes are characterized by elimination of the hydroxy-isopropylidene group with an acetoxy or benzoyloxy group. © **1997 by John Wiley & Sons, Ltd.**

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KEYWORDS: fast atom bombardment mass spectrometry; collision-induced dissociation; taxanes; cationization

INTRODUCTION

More than 100 new compounds have been added to the taxane diterpenoid family[1] since the discovery of the anticancer activity of the natural product paclitaxel (also known as taxol (Bristol-Myers Squibb) in the literature).[2] Because of the potential usefulness of taxanes in the partial synthesis of paclitaxel and its analogues and the potential clinical activity of these analogues,[3,4] investigations will continue and more taxoids will be isolated. Their structural elucidation is now carried out with the help of mass spectrometry (MS), various multi-dimensional NMR techniques and x-ray crystallography.[5] The last two techniques are often time consuming. For rapid screening procedures, MS/MS has been used successfully.[6] Detailed mass spectral studies of paclitaxel have also been reported.[7] However, there has been no comprehensive study of the mass spectra of various taxanes.

In connection with our work on the isolation of natural analogues of paclitaxel from the Himalayan yew, *Taxus wallichiana*, we have isolated several normal taxanes and *abeo*-taxanes containing the oxetane ring or the exocyclic methylene group (**1–14**). This paper summarizes their mass spectral behaviour studied by fast atom bombardment (FAB) MS followed by collision-induced dissociation (CID) of their $[M + Li]^+$, $[M + Na]^+$ and $[M + NH_4]^+$ ions.

EXPERIMENTAL

The taxanes **1–14** were isolated from the Himalayan yew, *Taxus wallichiana*. The FAB matrix *m*-nitrobenzyl alcohol (NBA) was obtained from Janssen Chimica (Belgium). FAB mass spectra were recorded on a Jeol SX-102/DA-6000 double-focusing (reverse geometry) mass spectrometer (10 kV accelerating potential) using a 6 kV xenon beam (10 mA), with NBA as the matrix. LiCl-, NaCl- and NH_4Cl-saturated NBA gave the corresponding $[M + Cat]^+$ ions. For FAB measurements 1 μl of a chloroform solution of the taxane (1 mg in 0.1 ml) was mixed on the probe tip with 1 μl of the matrix.

The CID spectra were recorded on the same instrument using linked scans at constant B/E and the mass analysed ion kinetic energy (MIKE) technique.[8] Linked scans were also carried out to obtain neutral mass-loss (at constant $B/E(1 - E/E_0)^{\frac{1}{2}}$) and precursor ion (at constant B^2/E) spectra. All the linked scan and CID MIKE spectra reported here were recorded with helium in the collision cell. The pressure of helium (in the first field-free region collision cell for linked scans and in the second field-free region collision cell for CID MIKE) was adjusted so that the parent ion signal was reduced by 50%. Each linked scan and CID MIKE spectrum reported here is an average of 3–6 scans.

RESULTS AND DISCUSSION

The FAB mass spectra of the taxanes **1–14** invariably showed $[M + Na]^+$ ions, probably resulting from the salt impurities introduced during their isolation from the natural sources. As a result, the normal FAB mass spectra contained peaks corresponding to fragment ions arising from $[M + H]^+$ and $[M + Na]^+$ ions. Cationization with Li^+, Na^+ and NH_4^+ resulted in abundant $[M + Cat]^+$ ions. In order to avoid any ambiguity in the assignment of fragmentation pathways, the CID spectra of the cation adducts were recorded using B/E linked scanning (B/E = constant) or the MIKE technique.

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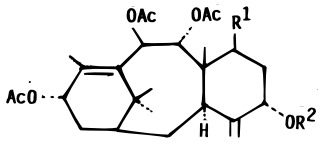
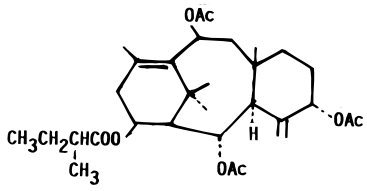


1 R^1 = H, R^2 = Ac
2 R^1 = OAc, R^2 = H
3 R^1 = OAc, R^2 = COCH:CHPh
4 R^1 = OAc, R^2 = $COCH_2CHNMe_2$ (Ph)



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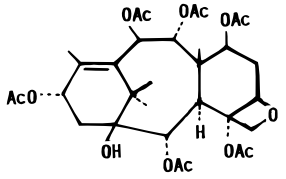

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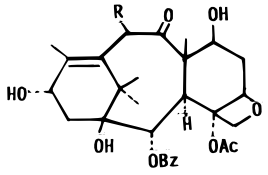
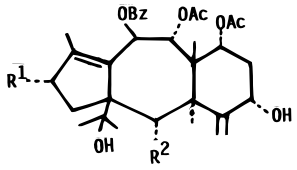
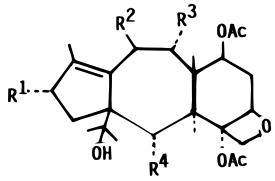


7 R = OH
8 R = OAc



9 R^1 = OH, R^2 = H
10 R^1 = OH, R^2 = OAc
11 R^1 = OAc, R^2 = H



12 R^1 = R^2 = OH, R^3 = R^4 = OAc
13 R^1 = R^2 = OAc, R^3 = R^4 = OBz
14 R^1 = OAc, R^2 = OH, R^3 = R^4 = OBz

The taxanes **1–14** consist of four groups, viz. (i) $\Delta^{4(20)}$-taxanes (**1–5**), (ii) taxanes containing an oxetane ring (**6–8**), (iii) $\Delta^{4(20)}11(15 \rightarrow 1)$*abeo*-taxanes (**9–11**) and (iv) $11(15 \rightarrow 1)$*abeo*-taxanes containing an oxetane ring (**12–14**). The $[M + Cat]^+$ ions of these taxanes undergo CID mainly by the losses of neutral molecules representing the various substituents in the molecule. Table 1 lists the neutral losses corresponding to the base peaks in the CID spectra of the $[M + Li]^+$, $[M + Na]^+$ and $[M + NH_4]^+$ ions. The CID mass

Table 1. Losses corresponding to the most intense peaks in the CID mass spectra of the $[M + Li]^+$, $[M + Na]^+$ and $[M + NH_4]^+$ ions of 1–14[a]

	Loss								
Compound	NH_3	(NH_3 + H_2O)	AcOH	(NH_3 + AcOH)	AcOLi	BzOH	(NH_3 + BzOH)	(NH_3 + BzOH + H_2O)	Others
1			+	×	○				
2			○, +	×					
3			○, +	×					
4			○, +	×					
5			○, +						x[b]
6			○, +	×					
7	x					○, +			
8	x		○, +						
9						○, +	×		
10						○, +		×	
11			○			+	×		
12		x	○, +						
13			○, +	×					
14		x	○, +						

[a] ○, $[M+Li]^+$; +, $[M+Na]^+$; ×, $[M+NH_4]^+$.
[b] $NH_3+CH_3CH_2CH(CH_3)COOH$.

spectra of the $[M + Li]^+$, $[M + Na]^+$ and $[M + NH_4]^+$ ions of representative examples from these groups are given in Figs. 1, 2 and 3, respectively. A partial listing of the major peaks in the CID mass spectra of the $[M + Li]^+$ and $[M + NH_4]^+$ ions of **1–14** is provided in Tables 2 and 3, respectively.

The CID data for the $[M + Na]^+$ ions are similar to those for the $[M + Li]^+$ ions. In general, the loss of a substituent in the vicinity of the double bond is favoured, apparently owing to the stabilization offered by the formation of a conjugated double bond. Accordingly, the substituent at C-10 is highly reactive and eliminates either H_2O, AcOH or BzOH. The presence of a carbonyl group at C-9 in **7** and **8** leads to the facile loss of NH_3 from $[M + NH_4]^+$ ion, leading to the base peak corresponding to the $[M + H]^+$ ion.

The presence of hydroxy groups in the molecule is best confirmed by examining the CID mass spectra of the $[M + NH_4]^+$ ions which show elimination of H_2O. For example, peaks corresponding to the loss of NH_3 and H_2O are prominent in **7**, **8** and **12–14**, while **9**, **10** and **11** give abundant peaks due to the loss of NH_3, BzOH and H_2O. These eliminations are suppressed in the CID mass spectra of the $[M + Li]^+$ and $[M + Na]^+$ ions as the alkali metal ions anchor at the hydroxy groups, hindering their elimination.

One of the characteristic reactions that the $[M + Li]^+$ or $[M + Na]^+$ ions of the acetyl derivatives (containing two adjacent acetoxy groups) undergo is the elimination of LiOAc or NaOAc. Peaks corresponding to these losses are observed in the CID spectra of the $[M + Cat]^+$ ions of **1–4** and **6**. These compounds contain two acetoxy groups at C-9 and C-10. It appears, therefore, that the anchimeric assistance of a neighbouring acetoxy group is required for the facile elimination of AcOLi or AcONa from $[M + Li]^+$ or $[M + Na]^+$ ion (Scheme 1). This is similar to the result reported previously in the CID of the $[M + Li]^+$ ions

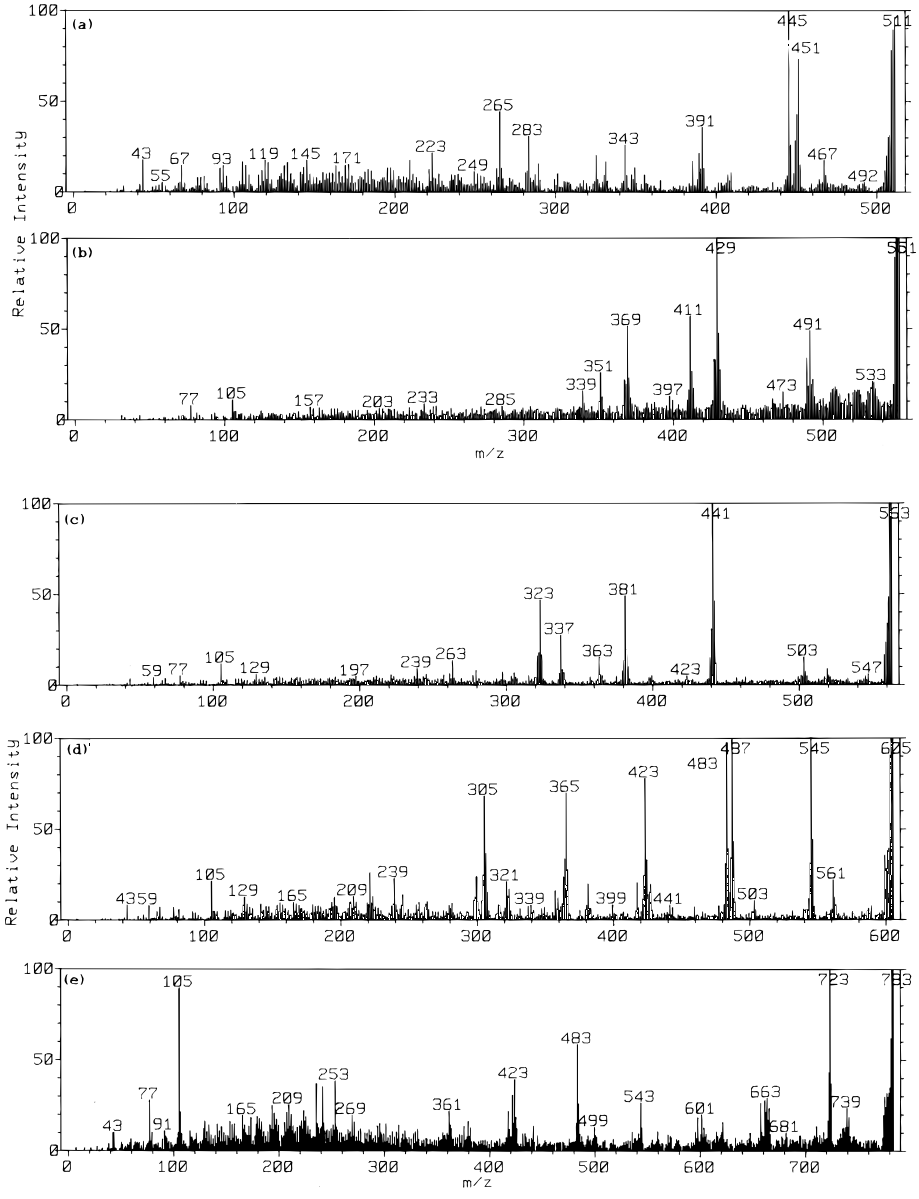


Figure 1. *B/E* CID spectra of the $[M + Li]^+$ ions of (a) **1** (*m/z* 511), (b) **7** (*m/z* 551), (c) **9** (*m/z* 563), (d) **11** (*m/z* 605) and (e) **13** (*m/z* 783).

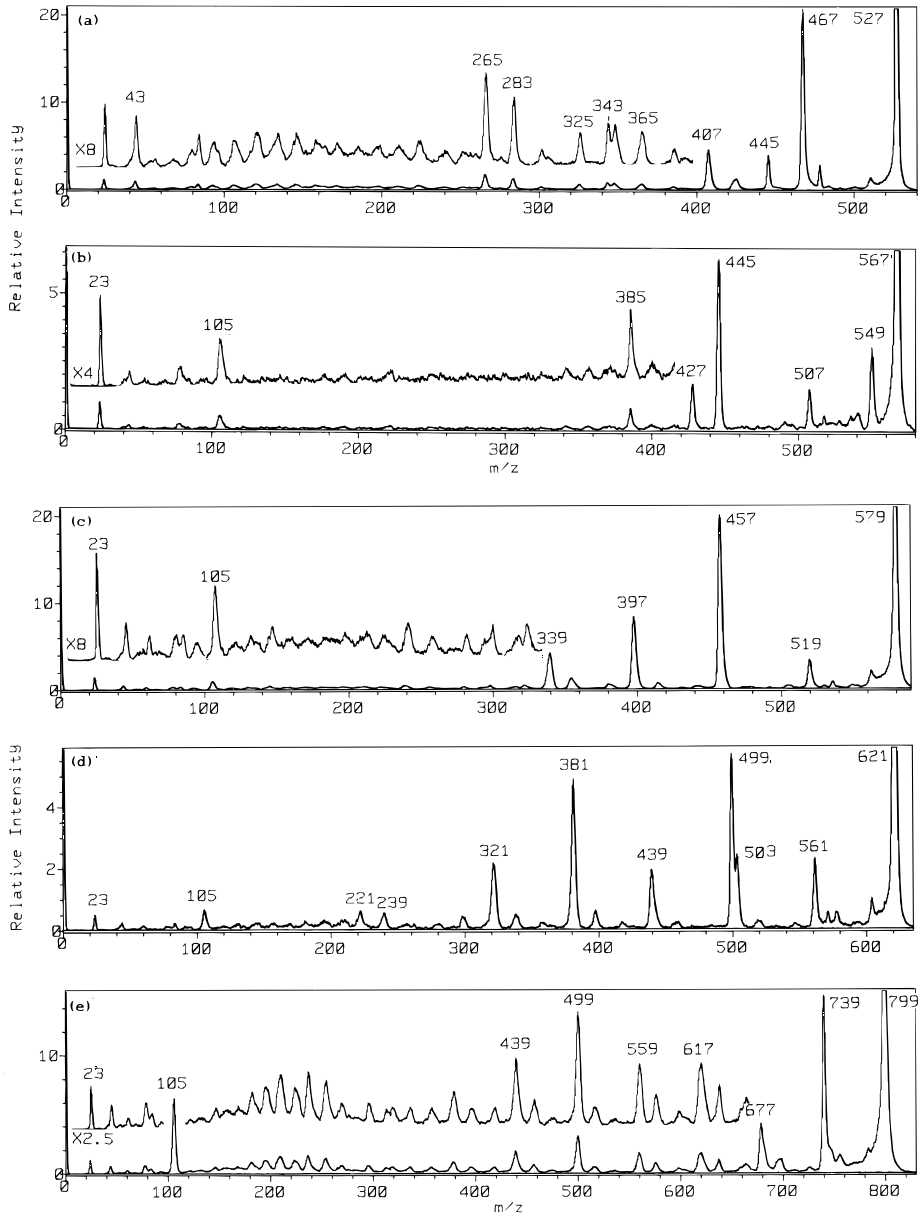


Figure 2. CID MIKE spectra of the $[M + Na]^+$ ions (a) **1** (*m/z* 527), (b) **7** (*m/z* 567), (c) **9** (*m/z* 579), (d) **11** (*m/z* 621) and (e) **13** (*m/z* 799).

of acetyl sugars.[9] This observation is useful in establishing the presence of acetyl groups at C-9 and C-10.

The ring system of taxoids is assigned routinely on the basis of NMR spectral results.[5] However, in the case of *abeo*-taxanes, often room temperature NMR spectra consist of broad peaks and careful temperature-dependent NMR spectral studies are required for the identification of this type of taxoid.[10] It was therefore of interest to see whether any characteristic fragmentation pattern could be established for the *abeo*-taxanes in the FAB tandem mass spectra, which are much faster to generate. The present data indicate that there is a characteristic fragmentation in the CID mass spectra of the $[M + Cat]^+$ ions of the *abeo*-taxanes **9**–**14**. This fragmentation, which is absent in the normal taxanes, involves the elimination of the C-1 hydroxypropyl group ($C_3H_7O^{\cdot}$) along with either an acetoxy or a benzoyloxy group. Accordingly, loss of ($C_3H_7O^{\cdot} + AcO^{\cdot}$) is observed in the CID mass spectra of the $[M + Cat]^+$ of **9**–**13** and ($C_3H_7O^{\cdot} + BzO^{\cdot}$) is lost in **14**. This elimination occurs directly from the $[M + Li]^+$ and $[M + Na]^+$ ions of **11**–**13**, whereas it is triggered only after the elimination of BzOH in **9** and **10** and AcOH in **14**. In the CID of the $[M + NH_4]^+$ ions, corresponding losses occur after the elimination of NH_3 and H_2O/BzOH. Direct loss of ($C_3H_7O^{\cdot} + AcO^{\cdot}$) from $[M + NH_4]^+$ is observed only in **11** and **13**.

There is no characteristic ring fragmentation which gives some indication of the position of the different substituents in a molecule. However, the CID spectra show ions formed by stripping off all the substituents. Thus the taxane skeleton with double bonds in place of substituents can be observed in the CID spectra of these taxanes (Table 4). However, the taxanes containing the oxetane ring do not give prominent identifiable taxane skeleton peaks. $\Delta^{4(20)}$*abeo*-Taxanes give these peaks at

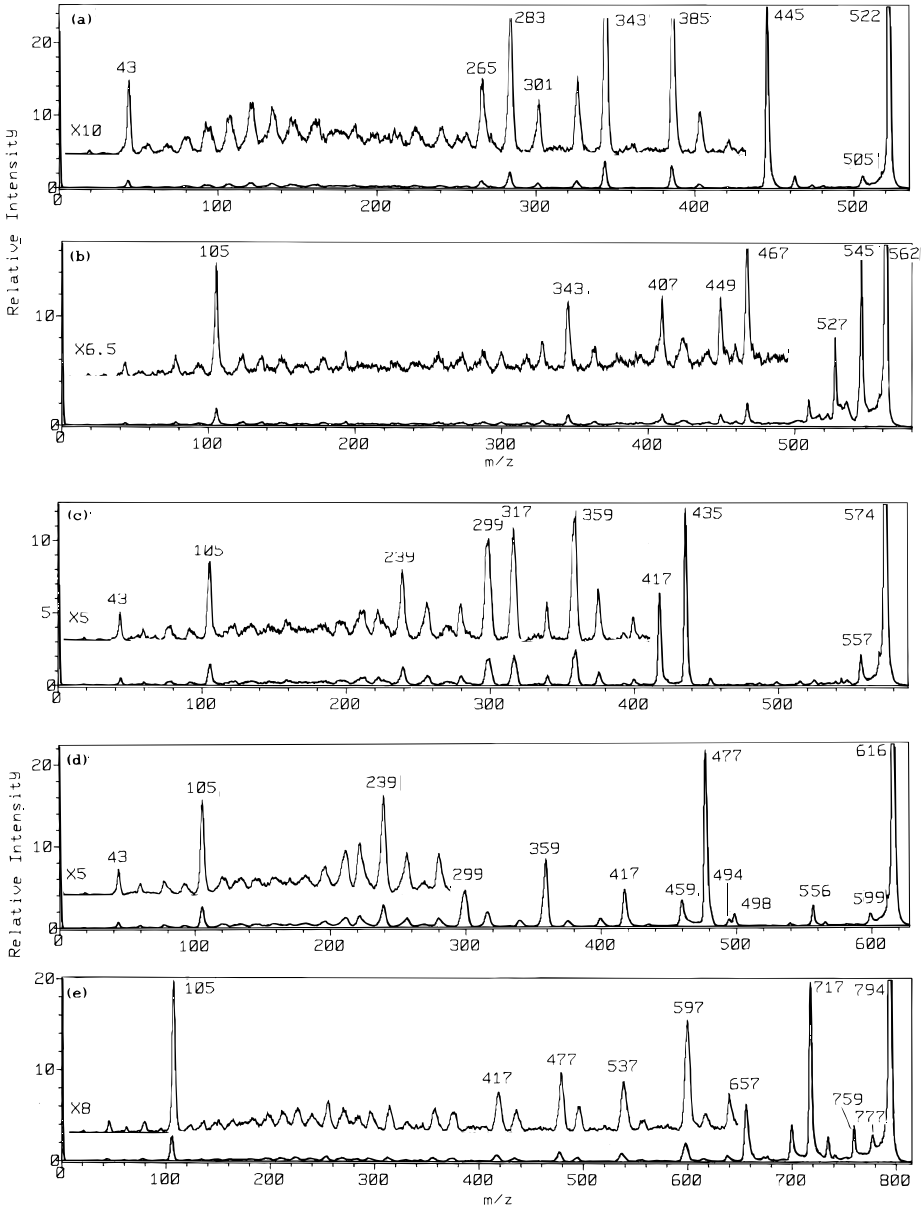
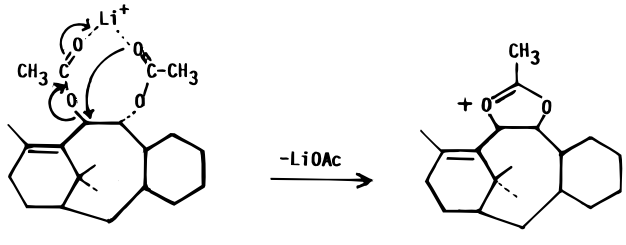


Figure 3. CID MIKE spectra of the $[M + NH_4]^+$ ions of (a) **1** (*m/z* 522), (b) **7** (*m/z* 562, (c) **9** (*m/z* 574), (d) **11** (*m/z* 616) and (e) **13** (*m/z* 794).

m/z 219/221, while $\Delta^{4(20)}$-taxanes show the corresponding peaks at *m/z* 261/263. Peaks are also observed for the corresponding hydroxylated taxanes at 18 and 36 mass units higher than the above values. From *B/E*, B^2/E and constant neutral mass-loss linked scan spectra, one of the possible sequences that leads to these ions is given in Scheme 2 for normal taxane **1** and in Scheme 3 for *abeo*-taxane **9**. Thus the *m/z* values of the peaks corresponding to the taxane skeleton also give an indication of the type of taxane involved.

In addition to abundant peaks corresponding to the elimination of AcOH and BzOH, the presence of acyloxy and benzyloxy groups is also characterized by abundant acyl (*m/z* 43) and benzoyl (*m/z* 105) ions. The presence of any specific substituent in the molecule other than OH, OAc and OBz is indicated by peaks



Scheme 1

Table 2. Partial *B*/*E* CID mass spectra of the $[M + Li]^+$ ions of 1–14; *m*/*z* (relative abundance, %)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
							Parent *m*/*z*							
Loss/ion	511	527	657	702	553	659	551	593	563	621	605	575	783	741
AcOH	451 (75)	467 (100)	597 (100)	642 (100)	493 (100)	599 (100)	491 (50)	533 (100)	503 (15)	561 (42)	545 (100)	515 (100)	723 (100)	681 (100)
AcOLi	445 (100)	461 (50)	591 (21)	636 (55)	—	593 (7)	—	—	—	—	—	—	—	—
AcOH + CH_2CO	409 (10)	425 (20)	555 (10)	594 (35)	451 (70)	557 (20)	—	—	—	—	503 (10)	—	681 (10)	—
2AcOH	391 (38)	407 (30)	537 (20)	582 (14)	433 (50)	539 (32)	—	473 (38)	—	—	485 (10)	455 (41)	663 (30)	—
$C_3H_7O^{\cdot}$ + $AcO^{\cdot}$	—	—	—	—	—	—	—	—	—	—	487 (98)	457 (20)	665 (24)	—
BzOH	—	—	—	—	—	—	429 (100)	—	441 (100)	499 (100)	483 (96)	—	661 (26)	—
BzOH + AcOH	—	—	—	—	—	—	369 (50)	—	381 (48)	439 (24)	423 (80)	—	601 (22)	449 (87)
BzOH + $C_3H_7O^{\cdot}$ + $AcO^{\cdot}$	—	—	—	—	—	—	—	—	323 (51)	381 (7)	365 (72)	—	543 (22)	—
AcOH + $C_3H_7O^{\cdot}$ + $BzO^{\cdot}$	—	—	—	—	—	—	—	—	—	—	—	—	—	501 (90)
m/*z* 105	—	—	—	—	—	—	10	18	12	10	23	—	90	25
m/*z* 43	18	17	5	6	18	6	2	2	4	6	8	16	8	5
Others			131 (20)	194 (38) 134 (50)		479 (20)	411 (60)				305 (70)	395 (42)	483 (60)	441 (87)

Table 3. Partial *B*/*E* CID mass spectra of the $[M + NH_4]^+$ ions of 1–14; *m*/*z* (relative abundance, %)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
							Parent *m*/*z*							
Loss/ion	522	538	668	696[a]	564	670	562	604	574	632	616	586	794	752
NH_3	505 (7)	521 (6)	651 (7)		547 (18)	653 (10)	545 (100)	587 (100)	557 (18)	615 (30)	599 (6)	569 (13)	777 (14)	735 (15)
NH_3 + AcOH	445 (100)	461 (100)	591 (100)	636 (100)	487 (5)	593 (100)	—	527 (55)	497 (2)	—	—	—	717 (100)	—
NH_3 + 2AcOH	385 (13)	401 (7)	443[b] (5)	576 (10)	385[c] (100)	533 (12)	—	467 (15)	—	—	—	—	657 (30)	—
NH_3 + 2AcOH + CH_2CO	343 (16)	359 (4)	401[d] (5)	534 (4)	343[e] (12)	491 (17)	—	—	—	—	—	—	—	—
NH_3 + H_2O	—	—	—	—	—	—	527 (50)	569 (10)	—	—	—	551 (100)	759 (20)	717 (100)
NH_3 + H_2O + AcoH	—	—	—	—	—	—	467 (13)	509 (22)	—	—	—	491 (5)	699 (22)	657 (8)
$C_3H_7O^{\cdot}$ + $AcO^{\cdot}$	—	—	—	—	—	—	—	—	—	—	498 (8)	—	676 (2)	—
NH_3 + BzOH	—	—	—	—	—	—	423 (2)	465 (7)	435 (100)	493 (35)	477 (100)	—	655 (15)	615 (2)
NH_3 + BzOH + H_2O	—	—	—	—	—	—	—	—	417 (50)	475 (100)	459 (15)	—	—	597 (5)
NH_3 + BzOH + AcOH	—	—	—	—	—	—	—	—	—	433 (40)	417 (21)	—	595 (10)	—
NH_3 + BzoH + $C_3H_7O^{\cdot}$ + $AcO^{\cdot}$	—	—	—	—	—	—	—	—	317 (18)	375 (60)	359 (35)	433[f] (12)	537 (8)	—
NH_3 + H_2O + AcOH + $C_3H_7O^{\cdot}$ + $BzO^{\cdot}$	—	—	—	—	—	—	—	—	—	—	—	—	—	477 (3)
m/*z* 105	—	—	—	—	—	—	10	18	12	32	12	—	17	7
m/*z* 43	4	2	2	3	4	6	1	2	4	12	3	3	1	—
Others			131 (5)	194 (44) 134 (13)					375 (10)				599 (10)	

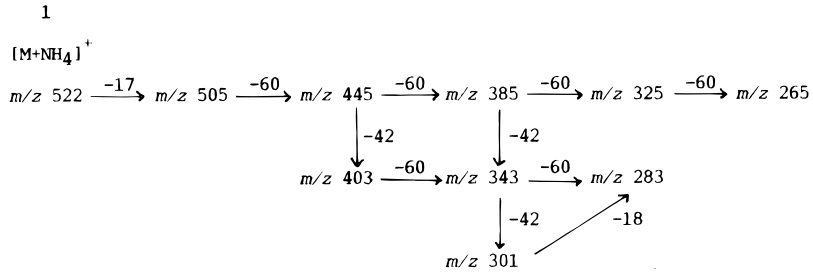
[a] *m*/*z* of $[M + H]^+$; $[M + NH_4]^+$ is not observed in the FAB mass spectrum with NH_4Cl-saturated NBA.
[b] NH_3 + AcOH + 102.
[c] NH_3 + AcOH + 148.
[d] NH_3 + AcOH + 102 + 42.
[e] NH_3 + AcoH + 148 + 42.
[f] $NH_3 + H_2O + C_3H_7O^{\cdot} + AcO^{\cdot}$.

Table 4. Abundances of the ions characteristic of the taxane ring in the *B/E* CID spectra of the $[M + Li]^+$ ions of 1–5 and 9–11

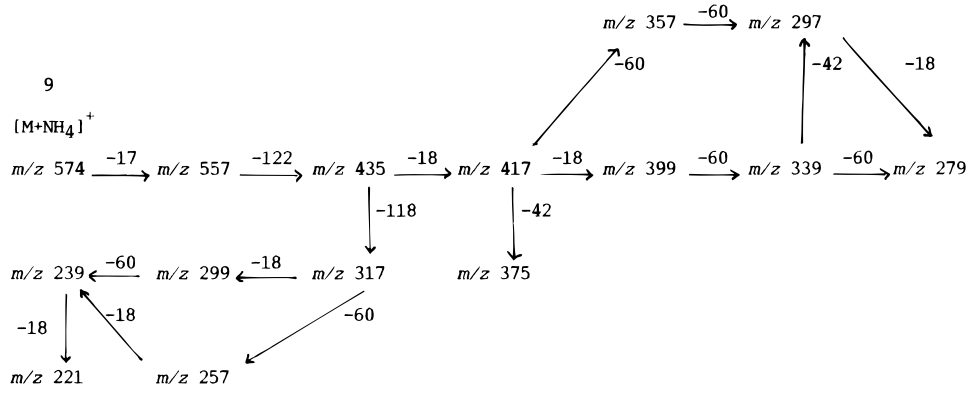
Compound	*m/z* (relative abundance, %)		
1	265 (46),	283 (32),	301 (10)
2	263 (18),	281 (26),	299 (16)
3	263 (22),	281 (15),	299 (8)
4	263 (11),	281 (15),	299 (8)
5	265 (48),	283 (20),	—
9	221 (6),	239 (10),	257 (5)
10	219 (7),	237 (18),	255 (12)
11	221 (26),	239 (24),	257 (8)

characteristic of these groups. For example, the cinnamoyl substituent in **3** gives rise to a peak at *m/z* 131 and the dimethylamino-containing substituent in **4** is characterised by the peaks at *m/z* 134 ($Ph(CH)=\overset{+}{N}Me_2$) and 194 ($H_2\overset{+}{O}COCH_2CH(Ph)NMe_2$). Because of the presence of the basic group, no $[M + NH_4]^+$ ion is formed from **4** under $[NH_4]^+$ cationization conditions. Instead, abundant $[M + H]^+$ ions are observed.

It is clear from the above results that FAB followed by MS/MS is a valuable tool for the identification of functional groups in taxanes and to some extent the type of taxane under investigation.



Scheme 2



Scheme 3

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