174. Fast-Atom-Bombardment Mass Spectra of Phloroglucinols from *Dryopteris* Ferns

by **Carl-Johan Widén***

Department of Pharmacognosy, University of Helsinki, Fabianinkatu 35, SF-00170 Helsinki

and **Heikki Pyysalo**

Finnish Customs Laboratory, Tekniikantie 13, SF-002150 Espoo

and **Tadeus Reichstein**

Institut für Organische Chemie, Universität Basel, St. Johanns-Ring 19, CH-4056 Basel

(20.VI.94)

Fast-atom-bombardment mass spectra of ten phloroglucinol derivatives containing two to six ring units were recorded. The mass spectral behavior of the compounds was quite similar under these conditions. Molecular-weight information was readily available as well as sequential structural data due to extensive fragmentation. Sequential alterations due to the rottlerone change or other analogous decomposition reactions proved to be almost lacking in fast-atom-bombardment mass spectrometry. The negative-ion spectra in general showed better signal-to-noise ratios and more distinct fragment ions.

Introduction. – The fern genus *Dryopteris* (Dryopteridaceae) contains phenolic compounds, which usually are designated *Dryopteris* phloroglucinols or acylphloroglucinols. *Ca.* 30 basic structures are found in which aromatic or isomeric hexa-2,5-dienone ring or more rarely hydropyrone rings are linked together *via* CH_2 bridges thus forming compounds with two to six rings [1–8]. Crude extracts or isolated substances especially from the male fern (*D. filix-mas*) have been used as effective anthelmintica, but, due to serious side-effects, they are no longer in use.

For structural elucidation of *Dryopteris* phloroglucinols, electron-impact mass spectrometry (EI-MS) has been widely employed during the last decades [7–17]. Under these conditions, however, extensive fragmentation occurs, and in the spectra of three- or four-ring compounds, the molecular-ion peak is often weak or lacking [11] [17]. Moreover, in the case of the compounds with three or four rings, thermal decompositions prior to ionization frequently occurs. Among these, the so-called rottlerone change and analogous reactions may lead to the formation of new products with altered molecular weights. For example, in EI-MS of unsymmetric phloroglucinol derivatives with three rings (X, Y, Z) disproportionation with exchange of the terminal rings may occur (*Scheme 1*) [11].

Scheme 1

$$2\ \ X{-}CH_2{-}Y{-}CH_2{-}Z \longrightarrow X{-}CH_2{-}Y{-}CH_2{-}X + Z{-}CH_2{-}Y{-}CH_2{-}Z$$

Moreover, by thermal genesis or/and EI-induced expulsion reactions new intra- and intermolecular linkages may be formed between originally unconnected ring moieties,

and two-ring products like X–CH_2–Z and X–CH_2–X could be observed in the spectra (Figs. 2 and 3 in [11]). This in turn can easily lead to misidentification of the original compound. Therefore, more 'soft' methods like chemical ionization (CI-MS) [18], field ionization (FI-MS) [19], and field desorption (FD-MS) [20] have been tried. With FD-MS, in the case of labile compounds containing three to six ring units, it was possible to greatly suppress the undesirable thermal reactions and to obtain the desired molecular-weight information alone with no or only little fragmentation depending on the experimental conditions. Another widely used soft ionization method in mass spectrometry is fast-atom-bombardment (FAB). Since its advent [21], it has had a remarkable impact on analyses of thermally labile, polar, or even ionic compounds as an easy-to-adopt method. Molecular-weight information is normally obtained, and, in many cases, also characteristic fragment ions are formed providing important structural information. In this paper, we report our results on ten *Dryopteris* phloroglucinols by positive- and negative-ion FAB-MS. Our goal was to find a readily available MS method giving distinct molecular-ion species and sequential fragment ions, but excluding artefacts formed by the rottlerone change or analogous decomposition reactions.

Materials and Methods. – The samples investigated consisted of ten substances isolated from rhizomes of different *Dryopteris* species (see below). For the *systematic IUPAC names* and *numbering* of the compounds studied, *cf.* [4] [6] (A: acetyl, B: butyryl, P: propionyl). All substances investigated proved to be pure and uniform (TLC, NMR, EI-MS). In some cases, traces of side-chain homologues including propionyl (P) were observed. The purity of the labile substances **37**, **38**, **23**, and **26** containing three to six rings in their molecules has been also tested by FD-MS [20].

1) *Albaspidin-AA* (**10-AA**): m.p. 164–166°, isolated from *D. hawaiiensis* [22].

2) *Trisalbaspidin-ABA* (= *filixic acid-ABA*; **19-ABA**): m.p. 167–169°, isolated from *D. wallichiana* [23].

3) *Tetraalbaspidin-ABBA* (= '*Methylene-bis-norflavaspidic acid-ABBA*', = '*Dryocrassin*'; **25-ABBA**): m.p. 209–211°, isolated from *D. wallichiana* [23].

4) *Pentaalbaspidin-BBBBB* (**37-BBBBB**): m.p. 194–196°, isolated from *D. aitoniana* (sample TR-1586) [4].

5) *Hexaalbaspidin-BBBBBB* (**38-BBBBBB**): m.p. 168–175°, isolated from *D. aitoniana* (sample TR-1587) [4].

6) *Flavaspidic Acid-BB* (**5-BB**): m.p. 158°, isolated from *D. 'pentheri'* (= *D. fadenii*) [15] [6].

7) *Trisflavaspidic Acid-BBB* (**23-BBB**): m.p. 171–172°, isolated from *D. aitoniana* (sample TR-1598) [4].

8) *Tetraflavaspidic Acid-BBBB* (**26-BBBB**): m.p. 170–171°, isolated from *D. aitoniana* (sample TR-1571) [4].

9) *Trisdesaspidin-BBB* (**21-BBB**): m.p. 145–148°, isolated from *D. 'austriaca'* (= *D. expansa*) [24].

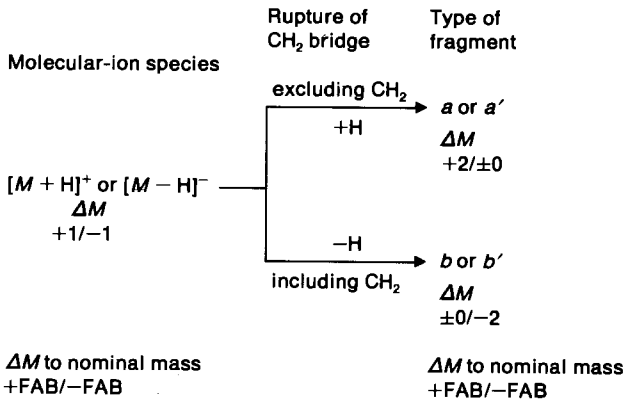
10) *Trisparaaspidin-BBB* (**20-BBB**): m.p. 148–152°, isolated from *D. submontana* [6].

FAB-MS was carried out using a *Jeol JMS-HX100* double-focussing instrument coupled with a *Jeol-DA5000* data system. Energy of bombarding Xe atoms was 6 KeV and the accelerating voltage of secondary ions 5 kV. The mass range of 35–2000 was scanned in 5 s. The resolution of the instrument was 3000. The samples were dissolved either straight in the matrix, 3-nitrobenzyl alcohol, or first in dichloromethane and then mixed with the matrix.

Results. – The overall behavior of the ten phloroglucinol derivatives in FAB mass spectrometry proved to be quite similar. In general, these compounds show less abundant but nevertheless distinct ion species, $[M + H]^+$ in positive FAB and $[M - H]^-$ in negative FAB. In addition, prominent fragment ions providing information about the sizes of the ring moieties are formed.

According to the interpretation of spectra, the main fragmentation process is a cleavage of the CH_2 bridge with concominant transfer of a H-atom from an OH group, located *ortho* to the bridge, to the adjacent ring as observed earlier in connection with EI-MS [11–13]. However, because of the existence of one additional H-atom in +FAB and absence of one in −FAB, the masses of the fragment ions differ by one unit from those observed in EI-MS, otherwise the spectra are rather similar. To facilitate the interpretation of spectra, the differences between the observed and nominal masses of the

Scheme 2. *A Simplified Fragmentation of a Phloroglucinol Derivative Showing the Type of Primary Fragments Formed and the Gain or Loss of H-Atoms as Compared to the Nominal Masses*



primary fragment ions are indicated in *Scheme 2* and are also inserted in the structural schemes presented.

The primarily formed fragment ions are designated a_n or a'_n when the CH_2 group is excluded, and b_n or b'_n when it is included in the fragment. The subscripts refer to the number of ring moieties in the corresponding fragment ions. For further details, see cleavage schemes of individual compounds below.

The substances of the present work are divided into three groups called the 'albaspidin group' (**10**, **19**, **25**, **37**, and **38**), the 'flavaspidic-acid group' (**5**, **23**, and **26**), and the 'aspidin group' (**21** and **20**). The side chains occurring are acetyl (A) or, most abundantly, butyryl (B) groups.

In the following, the positive-ion FAB (+FAB) and negative-ion FAB (−FAB) spectra of the individual substances are briefly discussed. The mass scale in the lower end is limited to m/z 180. Selected spectra are presented as an example from each group and are discussed in more detail. From the rest of the compounds, the observed primary fragments are given in the schematic structure drawings. The results obtained are compared with those of EI-MS and FD-MS earlier obtained on the same substances or their equivalents [11] [12] [17] [20].

The 'Albaspidin Group' (**10**, **19**, **25**, **37**, and **38**). The parent compound of this group, *albaspidin-AA* (**10-AA**), consists of two acetyl-filicinic-acid ring systems linked by a CH_2 group. In the rest of the substances, one to four butyryl-phloroglucinol rings connected by CH_2 groups are situated between the terminal acyl-filicinic-acid rings. Thus, substances of this group consists of symmetric molecules, if the acyl groups are identical as in the substances here studied, and only a_n and b_n fragments were formed in FAB.

Albaspidin-AA (**10-AA**). Both in the positive- and negative-ion spectrum (*Fig. 1*), distinct molecular-ion species for *albaspidin-AA* are present. In the +FAB spectrum, the protonated molecule $[M + H]^+$ appears at m/z 405 (base peak), and in the −FAB spectrum the deprotonated molecule $[M-H]^-$ is at m/z 403. The a_1 fragment in +FAB at m/z 197 is two mass units higher than its nominal mass, and in −FAB at m/z 195 (base peak), it equals to that. Accordingly, the b_1 fragment in +FAB at m/z 209 is equal to its nominal mass, and in −FAB at m/z 207 two mass units lower than that (*cf. Scheme 2*).

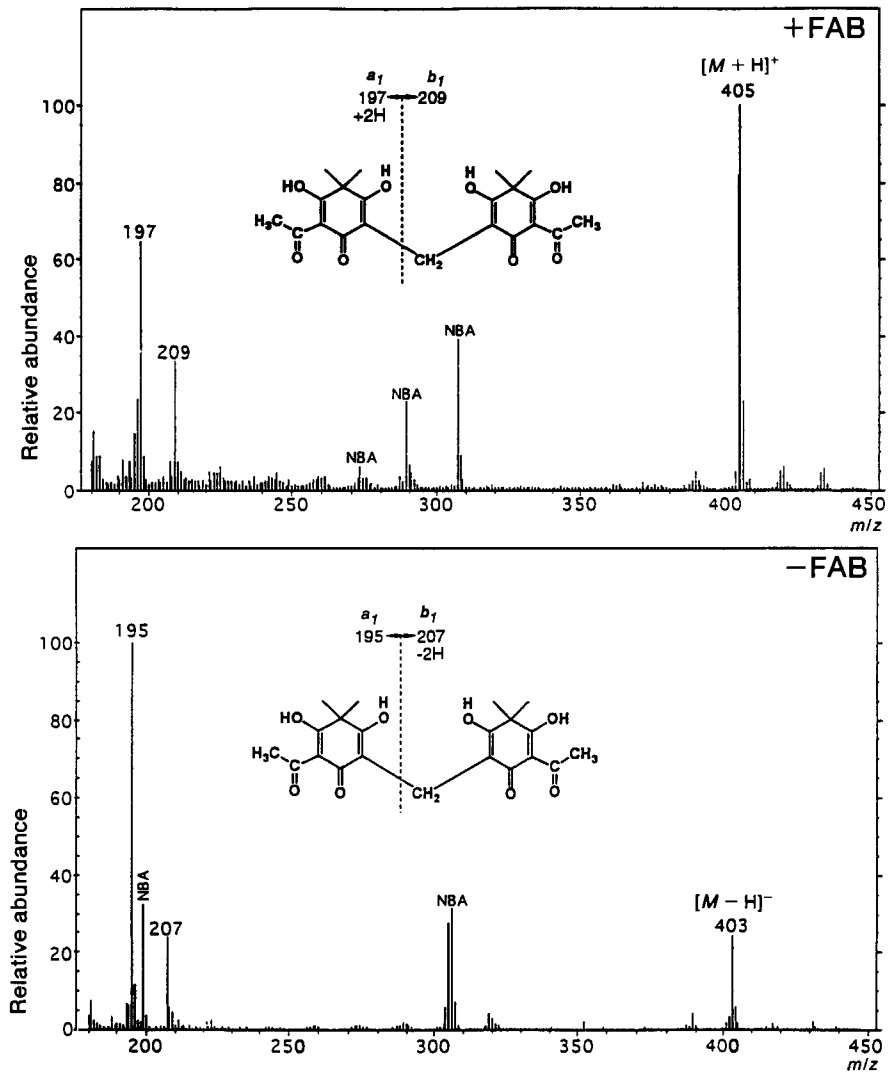


Fig. 1. *FAB-MS of albaspidin-AA* (**10-AA**) (peaks indicated by NBA originates from the matrix, 3-nitrobenzyl alcohol)

The EI-MS of **10-AA** and **10-BB** are discussed in [22] [12]. In both compounds, distinct molecular peaks at *m/z* 404 and 460, respectively, were found along with abundant fragment peaks.

Tetraalbaspidin-ABBA (**25-ABBA**, *Fig. 2*). The +FAB spectrum shows a rather weak $[M + H]^+$ peak at *m/z* 821 (*ca.* 2% relative intensity). The three-ring fragments at *m/z* 613 (a_3) and 625 (b_3) are missing. The two-ring fragment at *m/z* 417 (b_2) is clearly more abundant than the one at *m/z* 405 (a_2). However, these fragments also coincide with fragment ions formed from the two middle rings in this particular compound. In any case, *b* fragmentation seems to be more favorable in +FAB.

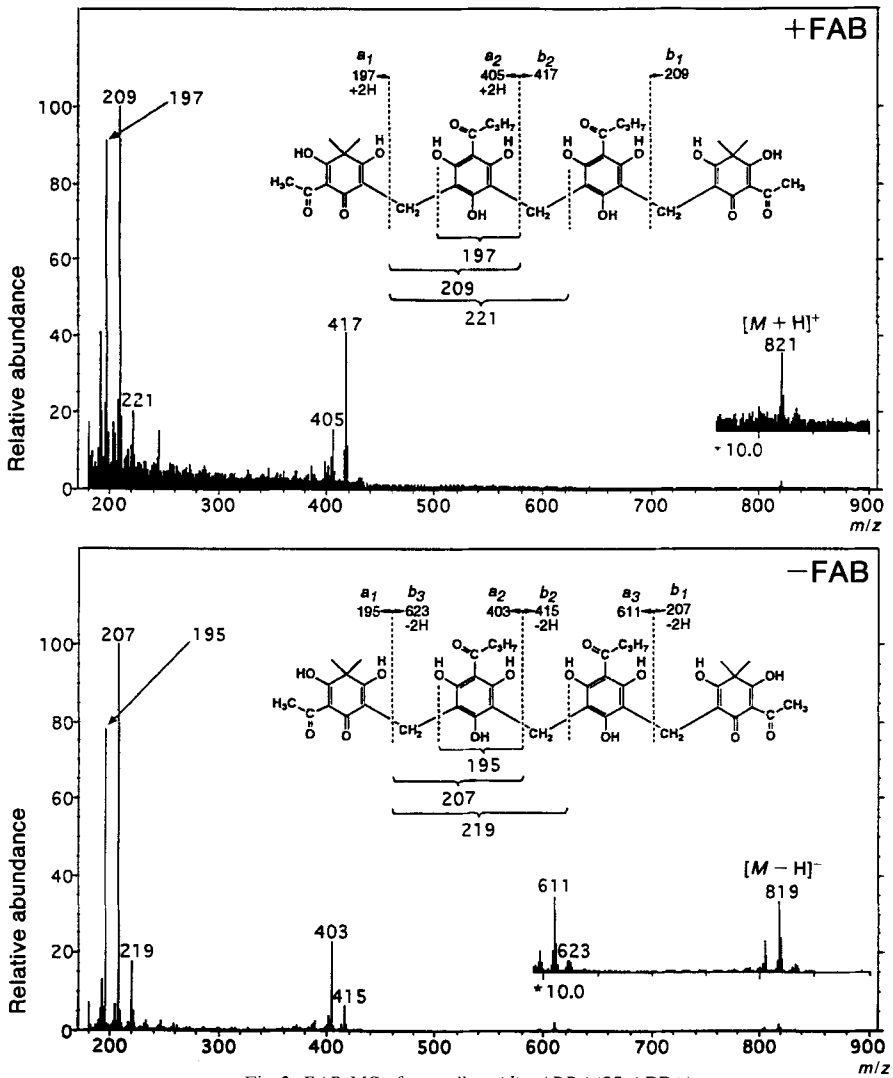


Fig. 2. *FAB-MS of tetraalbaspidin-ABBA* (**25-ABBA**)

The one-ring fragment at m/z 197 corresponds to the terminal fragment a_1 as well as to the fragment from the middle rings without CH_2 groups. However, in pentaalbaspidin-BBBBB (**37-BBBBB**) and hexaalbaspidin-BBBBBB (**38-BBBBBB**) with one and two additional middle rings of the same structure, respectively, the terminal a_1 fragment is at m/z 225 or at different mass as that of the fragment of the middle ring (197) (*cf. Schemes 5* and *3*). Therefore, we consider that the fragment at m/z 197 in **25-ABBA** is formed from both these fragment ions.

The fragment at m/z 209 (base peak) in turn could be due to the fragment b_1 or the fragment from the middle ring including one CH_2 group. In **37-BBBBB** and **38-BBBBBB** the corresponding peaks occur at m/z 237 and 225, respectively (*cf. Schemes 5* and *3*).

Apparently, the ion of m/z 209 is again formed from both fragments. The weak peak at m/z 221 arises from the fragment of the middle ring with both CH_2 groups included.

Also in the –FAB spectrum of **25-ABBA**, a weak $[M - H]^-$ ion (*ca.* 2% relative intensity) was detected at m/z 819. Moreover, another even less abundant (*ca.* 1% relative intensity) $[M - H]^-$ was observed at m/z 805. It may be due to the lower homologue APBA (**25-ABBA**). The three-ring fragment ions at m/z 611 (a_3) and 623 (b_3) (*ca.* 2 and 1%, respectively, relative intensity) are now visible. The two-ring peak at m/z 403 (a_3 *etc.*, see above) is now more abundant than that of 415 (b_2 *etc.*), indicating that the b-type fragmentation is unfavorable in –FAB.

In the FAB spectra of **25-ABBA** (*Fig. 2*), the ions of m/z 405 and 403 could at least partly be formed by albaspidin-AA (**10-AA**). In –FAB, there is another most interesting ion at m/z 611 (fragment a_3). This ion could, however, also be formed by trisalbaspidin-ABA (**19-ABA**) due to disproportionation reactions analogous to the rottlerone change (*cf.* [20]). The corresponding ion at m/z 613 was not detected in +FAB of **25-ABBA**.

However, as no such disproportionation products were found in the case of penta-albaspidin-BBBBB (**37-BBBBB**) (*Scheme 5*) with one more ring moiety, we consider their occurrence unlikely in FAB of **25-ABBa**.

In EI-MS, the molecular-ion peak of **25-ABBA** is very weak, and much thermal decomposition occurs; *inter alia*, the ions of m/z 612 (**19-ABA**) and 404 (**10-AA**), which are formed by disproportionation reactions analogous to the rottlerone change [17].

In FD-MS of **25-ABBA**, only M-like ions of the type $[M + H]^+$ were recorded at m/z 821, at an emitter current of 15 mA [20]. A minor M^+ ion at m/z 835 was considered to consist of the homologue **25-ABBP**. At elevated currents (21 mA and 23 mA), thermal rearrangements were detected in the case of the butyrylhomologue **25-BBBB**.

In the case of hexaalbaspidin-BBBBBB (**38-BBBBBB**; *Fig. 3* and *Scheme 3*), only a_n and b_n fragments are indicated. Both spectra of this sample show weak molecular-ion

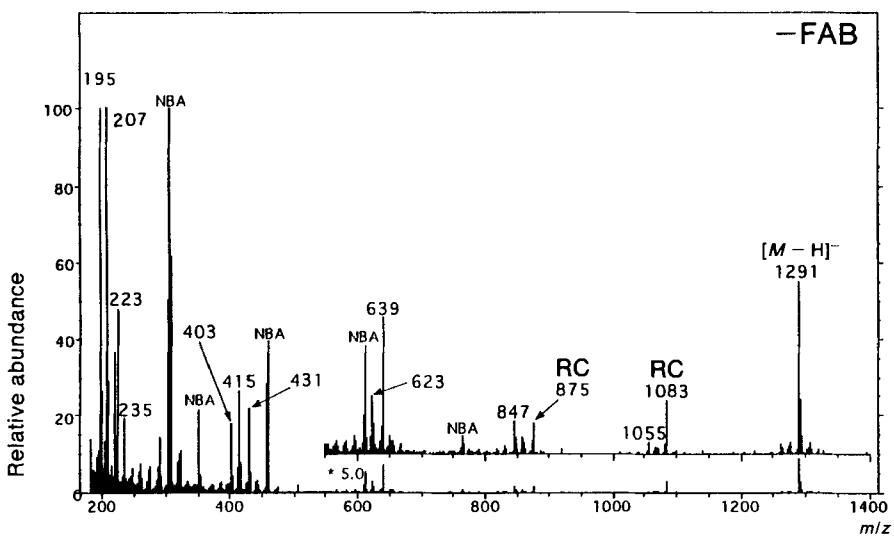
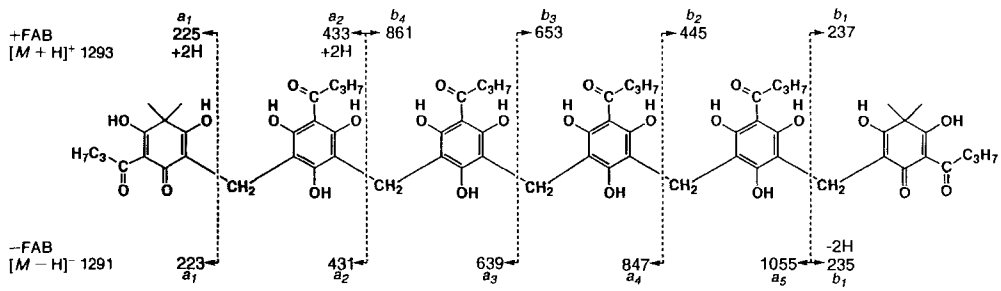


Fig. 3. *FAB-MS of hexaalbaspidin-BBBBBB* (**38-BBBBBB**). The peaks labelled with RC represents the products formed by the rottlerone change.

Scheme 3. *Hexaalbaspidin-BBBBBB* (**38-BBBBBB**) *with the Main Fragments Observed in FAB-MS*



species, confirming the molecular weight of 1292. The tendency in fragmentation is the same as in **25-ABBA**: in +FAB b_n and in −FAB a_n fragments are more abundant, and these fragments undergo further fragmentation as well.

Surprisingly, in the negative-ion spectrum, weak peaks appear at m/z 1083 and 875 (*Fig. 3*). These peaks cannot be interpreted according to the proposed fragmentation. They correspond to $[M - H]^-$ ions of pentaalbaspidin-BBBBB (**37-BBBBB**, mol. wt. 1084) and tetraalbaspidin-BBBB (**25-BBBB**, mol. wt. 876), respectively. However, according to the earlier results on our sample (TR-1587), it is chromatographically pure showing only one distinct spot in TLC [4], and in the FD-MS, only $M^{+\cdot}$ and $[M + H]^+$ signals at m/z 1292 and 1293, respectively, could be observed [20]. For the above reasons, these related substances hardly are present as impurities in our sample. Most probably, these ions are formed by decomposition reactions analogous to the rottlerone change, which are common under EI conditions; *i.e.*, pentaalbaspidin-BBBBB (**37-BBBBB**) is formed by expulsion of one and tetraalbaspidin-BBBB (**25-BBBB**) by expulsion of two of the central rings from the original hexaalbaspidin-BBBBBB (**38-BBBBBB**) and new bond formation between the terminal, non-linked ring systems, *cf.* [11] [20].

Apparently, interchange of the terminal rings also occurs, but, due to the symmetry of the molecule, this could not be verified. However, as no heat is needed in the ionization process, these reactions must be induced by the FAB. It is noteworthy that no corresponding ions at m/z 1085 and 877 were observed in the positive-ion spectrum of **38-BBBBBB**. However, the relative intensity of the ions in these mass regions were very weak (less than 1%).

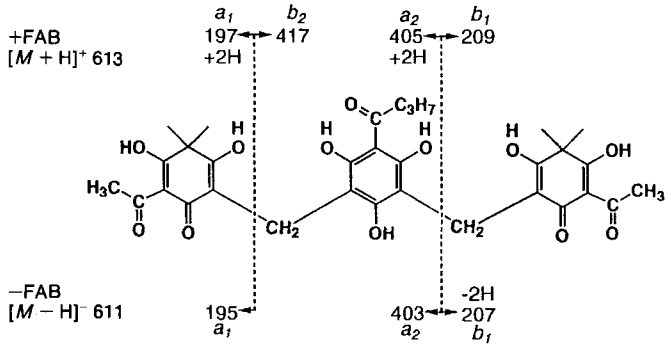
In EI-MS of **38-BBBBBB** as well as **37-BBBBB**, abundant fragmentations at the same masses as those of trisalbaspidin-BBB (**19-BBB**) were found. Moreover, the peaks at the highest masses were recorded at m/z 668 being the same as that of **19-BBB** [23].

The FAB-MS of **19-ABA** and **37-BBBBB** were not presented in the present work. Instead, schematic structural drawings were given in *Schemes 4* and *5* with the observed primary a_n and b_n fragments indicated.

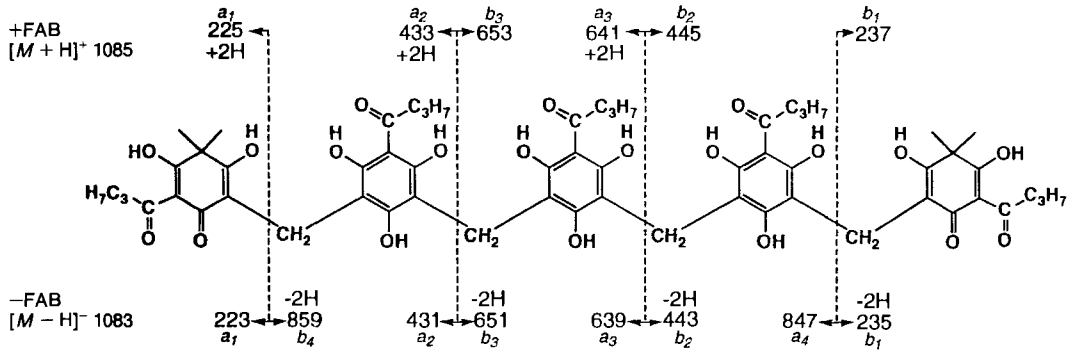
Trisalbaspidin-ABA (**19-ABA**). In the FAB-MS, distinct $[M + H]^+$ and $[M - H]^-$ peaks were recorded at *ca.* 19 and 13% relative intensity, respectively. As expected, the spectra were similar to those of tetraalbaspidin-ABBA (**19-ABBA**) with one more central unit (*Fig. 2*).

Pentaalbaspidin-BBBBB (**37-BBBBB**). The FAB-MS in turn, closely resembled those of hexaalbaspidin-BBBBBB (**38-BBBBBB**) with one more central ring moiety (*Scheme*

Scheme 4. *Trisalbaspidin-ABA* (**19-ABA**) *with the Main Fragments Observed in FAB-MS*



Scheme 5. *Pentaalbaspidin-BBBBB* (**37-BBBBB**) *with the Main Fragments Observed in FAB-MS*

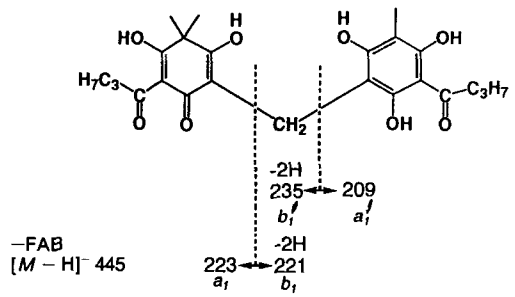


5). In the FAB-MS, weak molecular-ion peaks at *m/z* 1085 and 1083, respectively, were detected. It is noteworthy that no peaks, that could be attributed to the rottlerone change or analogous decomposition reactions, were observed. As in the previous cases *b* fragmentation seems to be preferable in +FAB and *a* fragmentation in −FAB. In FD-MS, only *M*-like ions of the type $M^{+\cdot}$ 1084 and $[M + H]^+$ 1085 were recorded at emitter current 20 mA [20].

The 'Flavaspidic-Acid Group' (**5**, **23**, and **26**). Here, the parent substance, flavaspidic acid-BB (**5-BB**), consists of a butyryl-filicinic unit connected with a CH_2 bridge to a 4-butyryl-2-methylphloroglucinol unit. In the two other substances **23** and **26**, one or two butyryl-phloroglucinol units, respectively, are situated between these rings. Because of the difference on one Me substituent in the terminal rings, the molecules of this group are unsymmetrical leading to the formation of both a_n and a'_n as well as b_n and b'_n fragment ions at different masses. All the substances investigated consist of pure butyryl homologues.

Flavaspidic Acid-BB (**5-BB**; *Scheme 6*). Only the −FAB spectrum was recorded. The spectrum is governed by the abundant molecular ion species $[M - H]^-$ at *m/z* 445. Furthermore, distinct fragment ions at *m/z* 209 (a'_1), 223 (a_1), 221 (b_1), and 235 (b'_1) were found.

Scheme 6. *Flavaspidic Acid-BB* (**5-BB**) *with the Main Fragments Observed in FAB-MS*



Trisflavaspidic Acid-BBB (**23-BBB**). The spectra of trisflavaspidic acid are presented in *Fig. 4*. In the positive-ion spectrum, the protonated molecule $[M + H]^+$ at m/z 655 is relatively abundant (*ca.* 25% relative intensity). The two-ring fragments at m/z 433 (a_2) and 445 (b_2') are quite distinct, but the other possible two-ring fragments from the other end of the molecule are lacking.

All possible one-ring fragments are present in **23-BBB**; the ions of 225 (a_1), 237 (b_1'), 209 (base peak; $a_2 - a_1 = b_2' - b_1'$), and 221 ($b_2' - a$) are discussed in connection with the +FAB spectrum of tetraalbaspidin-ABBA (**19-ABBA**, *Fig. 2*). The ion at m/z 211 (a_1') is formed from the third terminal ring moiety.

In the negative-ion spectrum, the deprotonated molecule ion $[M - H]^-$ is observed at m/z 653 (*ca.* 6% relative abundance). This time, the fragment at m/z 429 (b_2) represents the left side of the molecule, and both the fragments at m/z 431 (a_2) and 417 (a_2') are present, although weak. All the fragment ions containing one ring moiety are present as well.

In EI-MS of trisflavaspidic-acid BBB (**23-BBB**), the spectrum is dominated by abundant fragment ions [11]. The M^+ at m/z 654 is very weak as are those of the well known thermal recombination products **19-BBB** (M^+ 668) and **10-BB** (M^+ 460).

In the +FAB spectrum, b' fragmentation prevails, but, in the –FAB spectrum, no clear differences between a and a' as well as between b and b' fragmentation could be observed.

In FD-MS of **23-BBB**, abundant decomposition products were found as well [20]. Even at low emitter current (15 mA), minor peaks at m/z 446 and 447 were observed in addition to M^+ and $[M + H]^+$ signals at m/z 654 and 655, respectively. The former ones are most likely due to M^+ and $[M + H]^+$ of flavaspidic acid-BB (**5-BB**) formed by expulsion of the central ring in **23-BBB** and new bond formation between the two terminal rings [20]. At elevated emitter currents (18 mA), still other recombination products as well as other fragments were found. However, in FAB-MS of **23-BBB**, no products formed by rottlerone change or analogous rearrangements were observed (*Fig. 4*). All fragments may be attributed to sequential cleavage of the CH_2 bridges followed by H rearrangements.

Tetraflavaspidic Acid-BBBB (**26-BBBB**; *Scheme 7*). The positive-ion spectrum shows a weak protonated molecule $[M + H]^+$ at m/z 863 (*ca.* 1% relative intensity) and b' fragmentation prevails as in the case of **23-BBB**. In the negative-ion spectrum, a distinct

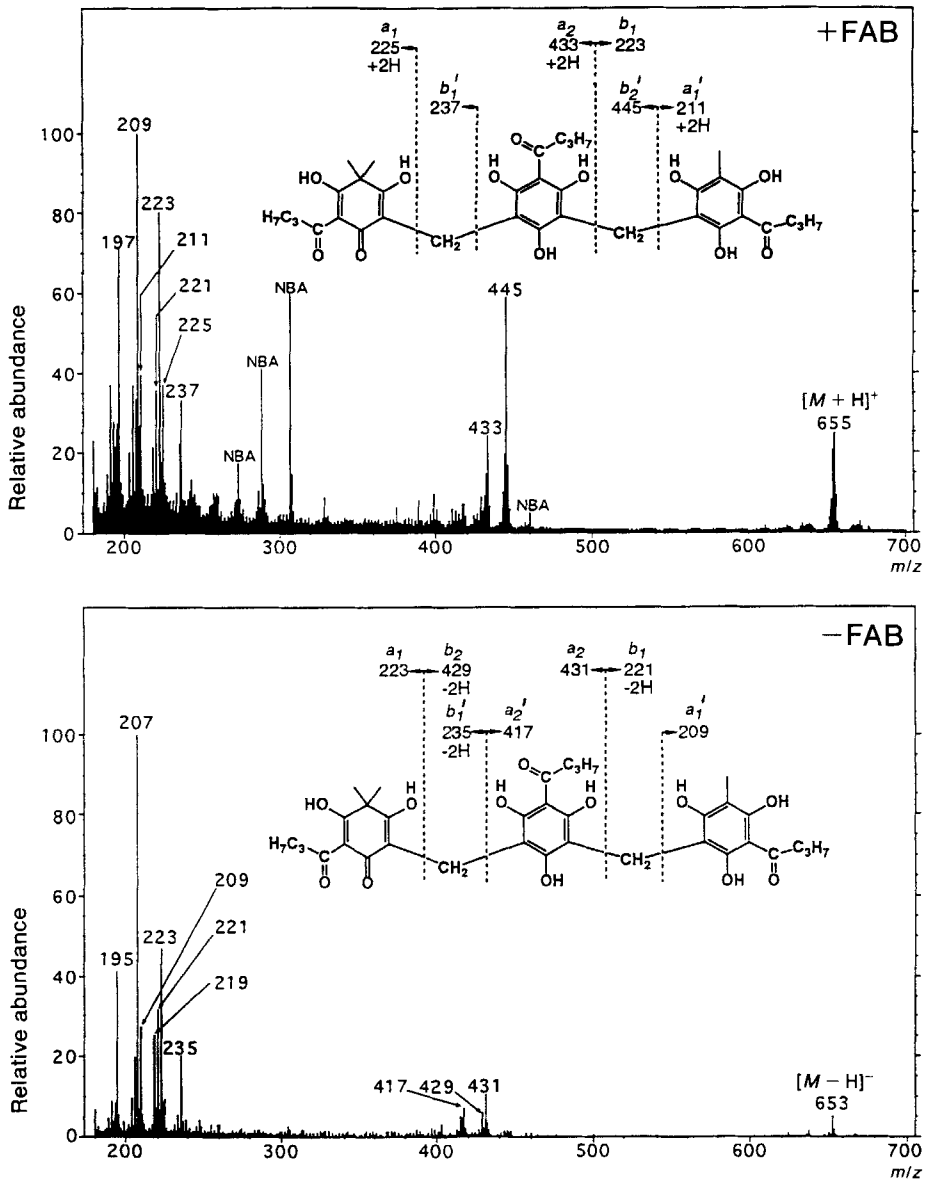
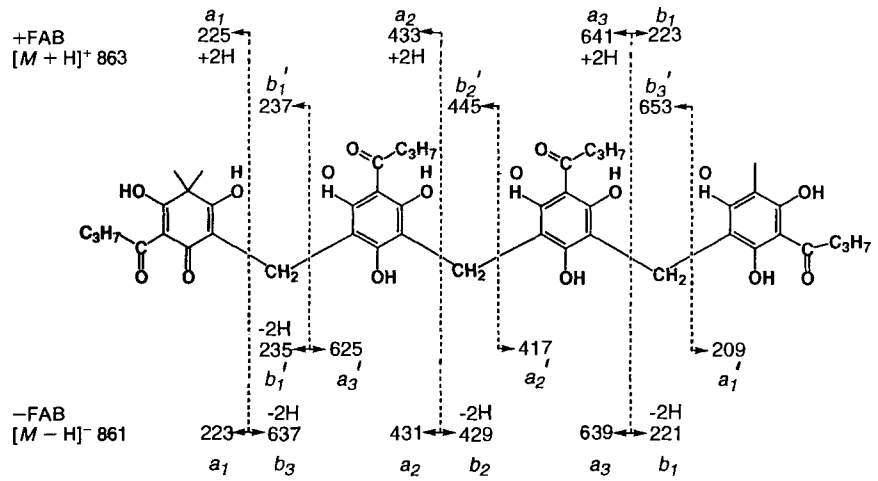


Fig. 4. *FAB-MS of trisflavaspidic acid-BBB* (**23-BBB**)

$[M - H]^-$ at m/z 861 is observed with *ca.* 17% relative intensity. No clear differences in abundances of a and a' as well as b and b' fragmentations were observed.

As probably expected the EI-MS of **26-BBBB** is very similar to that of **23-BBB** showing pronounced fragmentation. The weak peaks at the highest masses were detected at m/z 654 (**23-BBB**) and 668 (**19-BBB**) [23].

Scheme 7. *Tetraflavaspidic Acid-BBBB* (**26-BBBB**) *with the Main Fragments Observed in FAB-MS*



In FD-MS at 19-mA emitter current, distinct $M^{+\cdot}$ and $[M + H]^+$ signals were found at m/z 862 and 863, respectively, as well as expected thermal recombination products: **23-BBB** ($M^{+\cdot}$ 654 and $[M + H]^+$ 655) and **19-BBB** ($M^{+\cdot}$ 668, $[M + H]^+$ 669) [20]. At elevated emitter currents (21 mA), some rottlerone change (exchange of terminal rings), producing tetraalbaspidin-BBBB (**25-BBBB**) ($M^{+\cdot}$ at m/z 876), was additionally observed.

The 'Aspidin Group' [21] [20]. The parent compounds of this group, desaspidin-BB (**8-BB**) and paraaspidin-BB (**7-BB**), are monomethyl ethers of norflavaspidic acid-BB (**4-BB**) and flavaspidic acid-BB (**5-BB**), respectively (*cf.* [3] [4]). In the two substances of this group studied here, **21-BBB** and **20-BBB** one butyryl-phloroglucinol ring connected by CH_2 bridges is situated between the rings of the parent compounds. The methyl ether is located in the terminal aromatic ring. The structures of the molecules in this group are also unsymmetric providing different a_n and a_n' as well as b_n and b_n' fragment ions as in the 'flavaspidic-acid group'.

Trisdesaspidin-BBB (**21-BBB**). Both the positive- and negative-FAB spectra of **21-BBB** (*Fig. 5*) are almost identical to those of trisflavaspidic acid-BBB (**23-BBB**; *Fig. 4*). The sole differences in +FAB are slight differences in the relative abundances of the ions at m/z 209 ($a_2 - a_1 = b_2' - b_1'$) and 223 (b_1), and in –FAB small differences in the relative abundances of the ions at m/z 417 (a_2') and 429 (b_2). However, these two substances are isomeric compounds ($C_{35}H_{42}O_{12}$) and differ only in the position of the Me groups in the third ring.

As in the case of **23-BBB**, the EI-MS of **21-BBB** is characterized by abundant fragmentations [11]. Less frequently (*ca.* 1%), also products formed by the rottlerone change and other thermal recombination products occur (m/z 668 (**19-BBB**) and 446 (**5-BB**)). The molecular-ion peak $M^{+\cdot}$ at m/z 654 is very weak (*ca.* 1% relative intensity at 12 eV).

However, in FAB-MS of **21-BBB**, only sequential fragmentations were detected (*Fig. 5*). No FD-MS was recorded of **21-BBB**, but a FI-MS spectrum was kindly provided

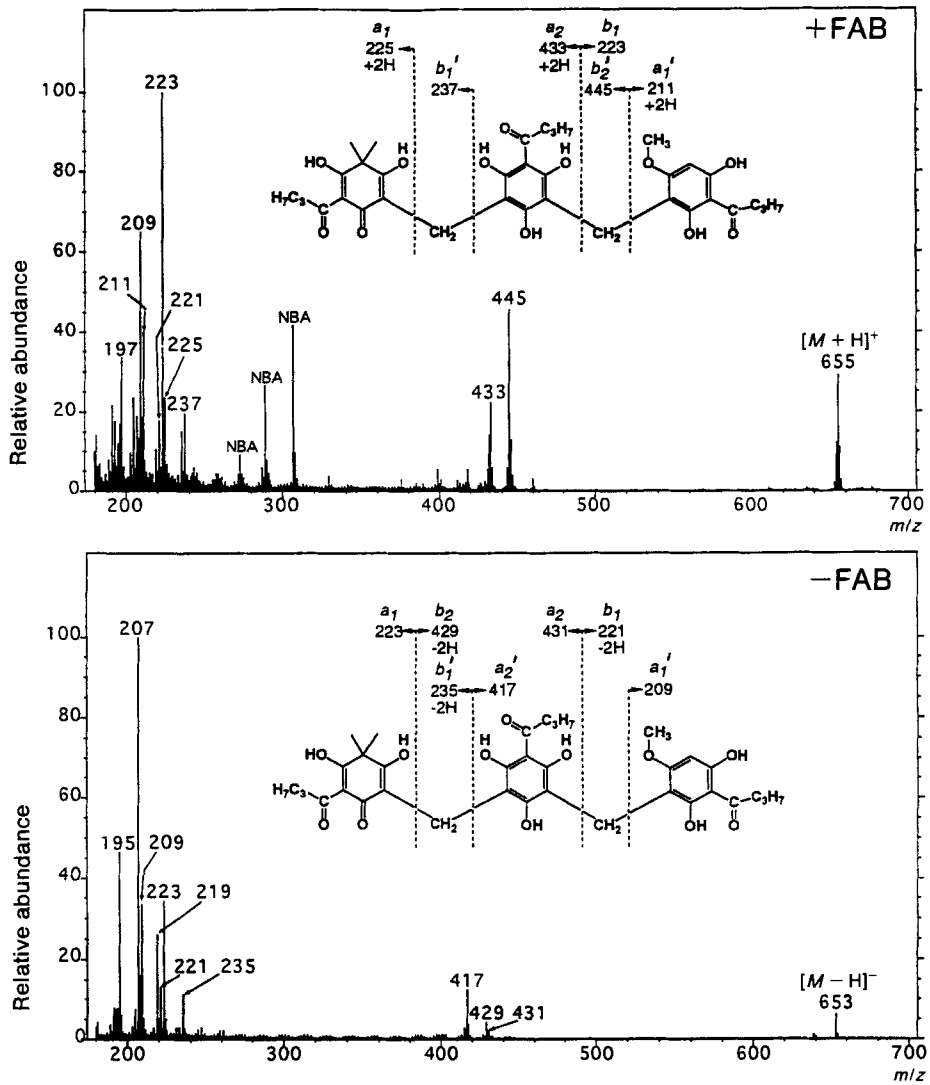


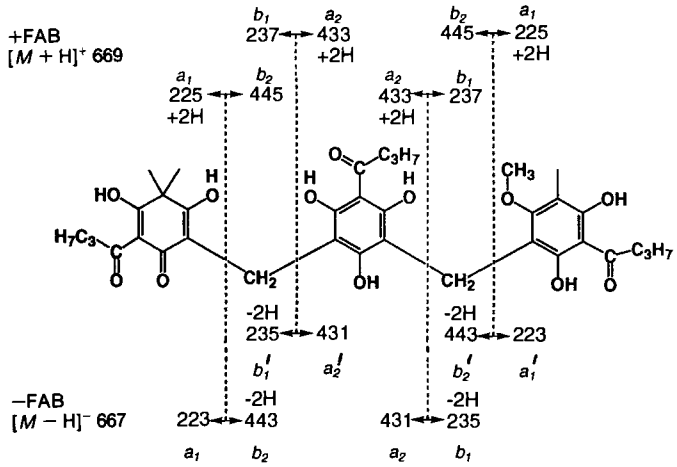
Fig. 5. *FAB-MS of trisdesaspidin-BBB* (**21-BBB**)

by the late *P. Brown* [19]. It showed $M^{+\cdot}$ at m/z 654 and a distinct signal at m/z 668, apparently due to **19-BBB** formed by rottlerone change.

Trisparaaspidin-BBB (**20-BBB**; *Scheme 8*). In the positive-ion spectrum, the $[M + H]^+$ occurs at m/z 669, and in the negative-ion spectrum $[M - H]^-$ is observed at m/z 667.

The terminal rings of **20-BBB** are isomeric and differ only in the position of one Me group (same masses). Therefore, the masses of the a_n ions are equal to those of the corresponding a'_n ions, and those of the b_n ions equal to those of the b'_n ions. Accordingly,

Scheme 8. *Trisparaaspidin-BBB* (**20-BBB**) *with the Main Fragments Observed in FAB-MS*



it is impossible to state which kind of fragmentation is more favorable. However, in +FAB, b_n and/or b'_n fragments dominate, whereas a_n and/or a'_n fragment prevail in −FAB as in the 'albaspidin group'. Also in the EI-MS of **20-BBB**, pronounced fragmentation and some thermal disproportionation were observed. In this case, the ion at m/z 460 ($C_{25}H_{32}O_8$) could be tentatively attributed both to albaspidin-BB (**10-BB**) or/and methylene-bis(aspidinol-BB) (**14-BB**; consisting of two terminal third rings connected by a CH_2 bridge; *cf.* [3] [4]). These are isomeric compounds.

Whether rottlerone change (exchange of the terminal rings) also occurred in FAB could not be decided, because **19-BBB** and **20-BBB** are isomeric compounds. However, in FAB of **20-BBB** (*Scheme 8*), only fragments caused by sequential fragmentation were found as in the case of the structurally related **23-BBB** and **21-BBB**.

Discussion. – FAB Mass spectrometry proved to be a most promising technique particularly in the analysis of the sensitive phloroglucinol derivatives with three to six ring moieties. Reliable information of molecular weights is readily available. Moreover, *via* rupturing CH_2 bridges, extensive fragment ions are formed producing sequential structural data. Using both positive- and negative-ion spectra, complementary fragments are obtained in many cases. The negative-ion spectra show in general better signal-to-noise ratios, more distinct fragment ions and unambiguous molecular-ion species.

In the 'albaspidin group' consisting of symmetric molecules, b_n fragment ions including terminal CH_2 groups dominate in +FAB, and a_n fragments excluding these groups in −FAB.

On the other hand, in the 'flavaspidic-acid group' as well as in trisdesaspidin-BBB (**21-BBB**; isomeric with trisflavaspidic acid-BBB (**23-BBB**)), all consisting of unsymmetric molecules, two different kinds of both a_n and b_n fragment ions occur at different masses. In +FAB, b'_n fragment ions prevail, but in −FAB no clear differences between the relative intensities of a_n and a'_n as well as of b_n and b'_n ions appear to exist.

The systematics in the fragmentation enables one to deduce the differences of ring substituents from the masses of fragment ions. However, as found in connection with

isomeric substances of the same elemental composition (**21-BBB** and **23-BBB**), the results of FAB spectrometry should always be combined with those of NMR and TLC to ensure correct identification of each compound. The troublesome rottlerone change and/or analogous reactions, nuisance in EI-MS (also occurring in FD-MS), do not cause problems in FAB-MS. This phenomenon was observed only in the −FAB spectrum of hexaalbaspidin-BBBBBB (**38-BBBBBB**), but not in the +FAB spectra of this or the other compounds.

In contrast to EI-MS and FD-MS, the labile, polar substances **21**, **23**, and **26** gave only $[M + H]^+$ and $[M - H]^-$ species as well as sequential fragments in FAB-MS. This may be due to the fact that no detrimental heat is needed for ionization of the samples as compared with the two other methods discussed.

In the light of the above facts, we consider that FAB-MS suits well to the aim stated in the *Introduction*: distinct molecular-species ions and virtually only sequential fragment ions are formed.

We thank Mr. *P. Ottoila* and Prof. *J. Taskinen*, Orion Research Center, Orion-Pharmaceuticals, Espoo, Finland, for recording the FAB-MS and their help in the interpretation. We are also grateful to the late Prof. *P. Brown* for submitting the FI-MS of trisdesaspidin.

REFERENCES

[1] G. Berti, F. Bottari, *Progr. Phytochem.* **1968,** *1,* 589.
[2] A. Penttilä, J. Sundman, *J. Pharmac.* **1970,** *22,* 393.
[3] J. v. Euw, M. Lounasmaa, T. Reichstein, C.-J. Widén, *Studia Geobotanica (Trieste)* **1980,** *1,* 275.
[4] J. v. Euw, T. Reichstein, C.-J. Widén, *Helv. Chim. Acta* **1985,** *68,* 1251.
[5] M. Coşkun, A. Sakushima, S. Nishibe, S. Hisada, *Chem. Pharm. Bull.* **1982,** *30,* 4102.
[6] C.-J. Widén, H. K. Widén, G. Vida, T. Reichstein, *Bot. Helv.* **1991,** *101,* 77.
[7] C.-J. Widén, P. Äyräs, T. Reichstein, *Ann. Bot. Fennici* **1992,** *29,* 41.
[8] C.-J. Widén, P. Äyräs, K. Neuvonen, T. Reichstein, *Ann. Bot. Fennici* **1993,** *30,* 285.
[9] M. Lounasmaa, A. Karjalainen, C.-J. Widén, A. Huhtikangas, *Acta Chem. Scand.* **1971,** *25,* 3428.
[10] M. Lounasmaa, A. Karjalainen, C.-J. Widén, A. Huhtikangas, *Acta Chem. Scand.* **1971,** *25,* 3441.
[11] M. Lounasmaa, C.-J. Widén, T. Reichstein, *Helv. Chim. Acta* **1971,** *54,* 2850.
[12] M. Lounasmaa, A. Karjalainen, C.-J. Widén, A. Huhtikangas, *Acta Chem. Scand.* **1972,** *26,* 88.
[13] M. Lounasmaa, C.-J. Widén, T. Reichstein, *Helv. Chim. Acta* **1973,** *56,* 1133.
[14] M. Lounasmaa, *Planta Med.* **1973,** *24,* 148.
[15] C.-J. Widén, R. B. Faden, M. Lounasmaa, G. Vida, J. v. Euw, T. Reichstein, *Helv. Chim. Acta* **1973,** *56,* 2125.
[16] C.-J. Widén, M. Lounasmaa, G. Vida, T. Reichstein, *Helv. Chim. Acta* **1975,** *58,* 880.
[17] C.-J. Widén, M. Lounasmaa, J. Sarvela, *Acta Chem. Scand., Ser. B* **1975,** *29,* 859.
[18] M. Lounasmaa, *Planta Med.* **1987,** *37,* 881.
[19] P. Brown, unpublished.
[20] W. J. Richter, F. Rashdorf, J. v. Euw, T. Reichstein, C.-J. Widén, *Helv. Chim. Acta* **1987,** *70,* 881.
[21] M. Barber, R. S. Bordoli, R. D. Sedgwick, A. N. Tyler, *J. Chem. Soc., Chem. Commun.* **1981,** 325.
[22] T. Patama, C.-J. Widén, *Phytochemistry* **1991,** *30,* 3305.
[23] C.-J. Widén, unpublished.
[24] C.-J. Widén, M. Lounasmaa, J. Sarvela, *Planta Med.* **1975,** *28,* 144.