A Mass Spectral Analysis of some Cucurbitacins Isolated from *Fevillea cordifolia*

Lyndon B. N. Johnson,[a] William J. Griffiths,[a] Earle V. Roberts,*[,a] Lister K. P. Lam,[b] John C. Vederas,[b] Colin J. Reid[c] and James A. Ballantine[c]

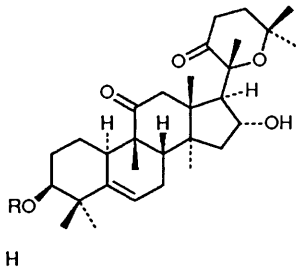
[a] *Department of Chemistry, University of the West Indies, Mona, Kingston 7, Jamaica*
[b] *Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2*
[c] *SERC Mass Spectrometry Service Centre, University College of Swansea, Singleton Park, Swansea SA2 8PP, Wales, UK*

An examination of the mass spectral behaviour of cordifolin A **1** (3β,16α-dihydroxy-20,25-epoxy-19(10→9β)*abeo*-10α-lanost-5-en-11,22-dione), 3-*O*-β-D-glucopyranosyl-6,1-β-D-glucopyranosidecordifolin A **2**, 22α-hydroxy-23,24-dehydrocordifolin A **3**, and 22-deoxo-23,24-dehydrocordifolin A **4**, isolated from *Fevillea cordifolia* (Cucurbitaceae), illustrates the diagnostic fragmentation pathways that occur in these cucurbitacins. Tandem mass spectrometry allowed the construction of a detailed fragmentation map of **1**. The structure elucidation of compounds **2–4** and their EI, CI and FAB MS as well as those of cordifolin A **1** are discussed.

Mass spectrometric studies have yielded a wealth of information in the structural characterization of triterpene skeletons, and in locating the functional groups present in these molecules.[1–6] Ionization techniques such as electron impact (EI),[1,2] chemical ionization (CI),[6] field desorption (FD),[7] fast atom bombardment (FAB),[8] and secondary ion (SI)[9] mass spectrometry (MS) have been extensively employed. The cucurbitacins, triterpenes which possess the biogenetically unusual 19(10→9β)*abeo*-10α-lanostane skeleton,[10,11] have evoked wide interest because of the host of biological activities that members of this series display.[12] Numerous reports have appeared in the literature[13–20] outlining the application of mass spectrometry to the structure determination of the cucurbitacins. However, very few have provided detailed fragmentation patterns for these compounds.[20] Recent examination of the seeds of *Fevillea cordifolia* L. (Cucurbitaceae) led to the isolation and structure elucidation of a novel pentacyclic triterpene, cordifolin A **1**.[21] Application of multi-pulse NMR methods proved to be essential to obtain the structure of **1** which displays extensive resonance overlap. Further investigation of the plant extract allowed us to purify three more cucurbitacins **2–4**. In addition to multi-pulse NMR methods, a number of mass spectral methods including EI, CI, FAB and tandem mass spectrometry (MS–MS) were utilized in the structural elucidation of these compounds. These results are presented in this paper.

Results and Discussion

Mass spectral cleavages which were important in the characterization of the cyclised side chain of cordifolin A **1** have been discussed earlier.[21] Cordifolin A exhibits mass spectral reactions in its tetracyclic framework similar to those observed in other functionalized triterpenes such as the hopanes,[1] lanostanes[22] and allobetulanes.[1] Thus, ring C scission, common among members of these series, was observed in the EI and FAB mass spectra of **1** (Schemes 1 and 2). The presence of the 11-oxo function renders ring C susceptible to cleavage, which probably proceeds *via* α-cleavage of the C(11)–C(12) bond followed by abstraction of a proton from C-7 and cleavage of the C(8)–C(14) bond to give a peak at *m/z* 173.

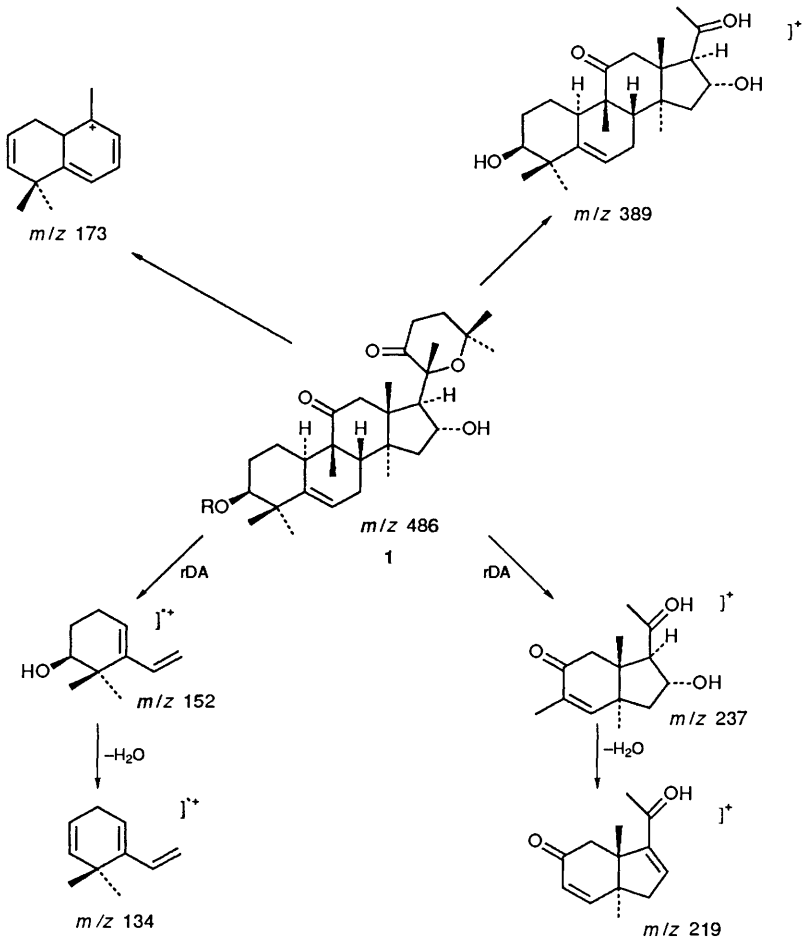


1 R = H
2 R = β-D-glucopyranosyl-6,1-β-D-glucopyranosyl

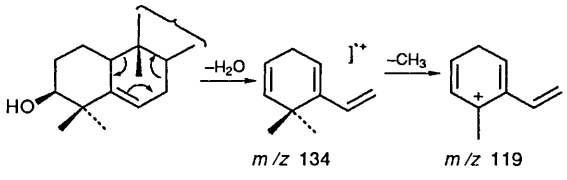
Ring B fissure in compound **1** was also prominent and can be accounted for by the occurrence of a retro Diels–Alder (rDA) mechanism, initiated by the Δ-5 olefinic moiety. The peaks which occurred at *m/z* 152.1183 ($C_{10}H_{16}O$) and *m/z* 237.1500 ($C_{14}H_{21}O_3$) were indicative of this cleavage and represented rings A and CD, respectively. Audier and Das[18] and, more recently, Gamlath *et al.*[16] have reported that cleavage *via* a rDA mechanism was very significant in the mass spectra of cucurbitacins which possessed the diosphenolic moiety. There were also, however, indications that the rDA mechanism was not limited to cucurbitacins which possess the diosphenolic moiety. In a study of the sweet and bitter cucurbitane glycosides of *Hemsleya carnosiflora*,[17] Tanaka and co-workers reported an rDA mechanism even though the diosphenolic substructure was absent from these compounds.

There were noticeable differences in the mass spectrum of **1** under the 'softer' ionizing conditions of FAB, wherein the dominance of a different fragmentation pathway was evident. Whereas the cleavages of the cyclized chain were very pronounced in the EI spectrum, the rDA reaction of ring B, driven by the Δ-5 function, acted as the principal source of the prominent ions observed in FAB. Thus, ring B fissure resulted in a fragment ion at *m/z* 135 [$(134 + H)^{\bullet+}$, 61%], which subsequently lost a methane molecule, to afford a fragment ion at *m/z* 119 (100%). This fragment ion at *m/z* 119 was previously observed by Gamlath and co-workers[23] in a study of compounds isolated from *Reissantia indica*, which possess the novel 19(10→9)*abeo*-8α,9β,10α-euphane skeleton.

An MS–MS[24] study of cordifolin A allowed the establishment of the parent–daughter ion relationships in this compound. Thus, the EI CA–MIKE (collision activated–mass-analysed ion kinetic energy) spectrum of the molecular ion (*m/z* 486) showed fragment ions which are the products of the primary fragmentary cleavages occurring in cordifolin A. The

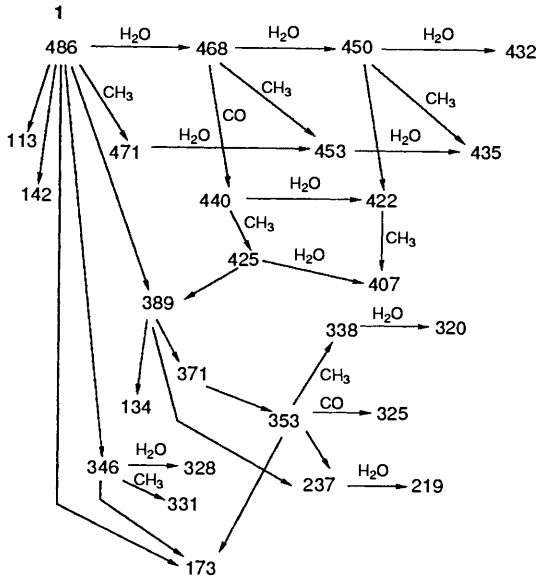


Scheme 1



Scheme 2

MS–MS information was interpreted as follows: facile loss of a molecule of water from the molecular ion yields *m/z* 468, while peaks at *m/z* 113 and 142 were the primary products of fissures which involved the cyclized side-chain. The fragment ion at *m/z* 389, and its satellites at *m/z* 371 and 353, represented the tetracyclic framework left after loss of the side-chain. The occurrence of primary skeletal fragmentations in the tetracyclic framework of cordifolin A was signified by the peak at *m/z* 173. It is interesting that no evidence of the rDA reaction, which involves the Δ-5 function and is suggested by the presence of daughter ions at *m/z* 152 and 134, was found. The rDA cleavage was therefore not a primary process in the fragmentation of the molecular ion. A similar study of the MIKE spectra of peaks at *m/z* 468, 450, 425, 353, 389 and 346 allowed the construction of a fragmentation map of cordifolin A (Scheme 3).



Scheme 3

Fourier transform IR spectroscopy (FTIR) of compound **2** suggests the presence of hydroxy, carbonyl and olefinic functionalities with absorption bands at 3220–3590, 1685 and 1630 cm^{-1}, respectively. Results obtained from the attached proton test (APT)[25,26] and the normal ^{13}C and ^{1}H NMR spectra of compound **2** are summarized in Table 1. Application of multi-pulse NMR techniques, such as direct ^{1}H, ^{1}H INADEQUATE,[27,28] long range and direct ^{1}H,^{13}C heteronuclear correlation spectra (COLOC[29] and HETCOR[30,31]), and one dimensional nuclear Overhauser effect (NOE) difference spectroscopy,[32,33] for the structural elucidation of **2** is analogous to that reported for cordifolin A **1**.[21] When **1** is used as a reference compound in the spectral analysis of compound **2**, close similarities are observed between the two molecules in rings B, C, D and E. Ring A provided the major point of difference. The C-3 resonance shows a glycosylation shift

Table 1 ^{13}C and ^{1}H NMR chemical shifts[a] of compound **2**

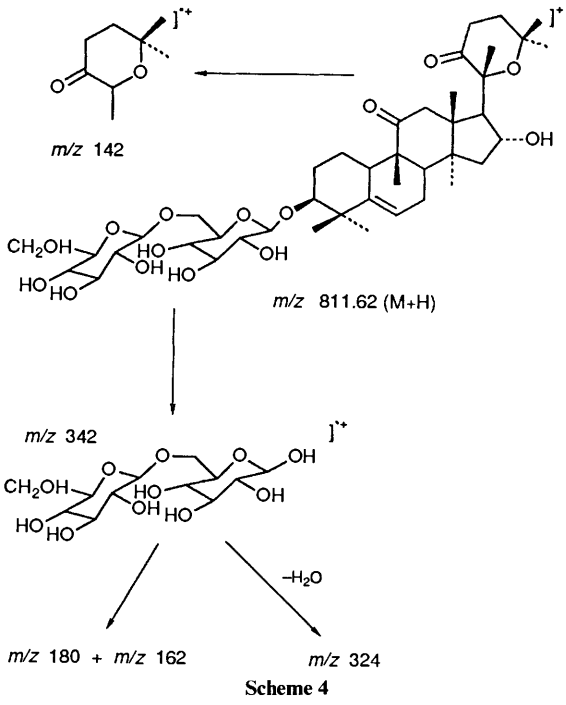
Carbon	Multiplicity	δ_C(ppm)	δ_H(ppm)
1	CH_2	22.48	1.32 (H_α), 1.58 (H_β)
2	CH_2	28.93	1.96 (H_α), 1.78 (H_β)
3	CH	87.59	3.46
4	C_q	42.62	
5	C_q	142.06	
6	CH	119.58	5.61 (d, *J* 5.6 Hz)
7	CH_2	24.78	1.96 (H_α), 2.33 (H_β)
8	CH	44.53	1.88
9	C_q	50.39	
10	CH	36.68	2.42
11	C_q	216.87	
12	CH_2	49.88	3.31 (H_α), 2.51 (H_β) (*J* 14.4 Hz)
13	C_q	49.31	
14	C_q	51.91	
15	CH_2	46.73	1.36 (H_α), 1.82 (H_β)
16	CH	71.53	4.41 (t, *J* 7.6 Hz)
17	CH	59.29	2.56 (d, *J* 7.4 Hz)
18	CH_3	20.37	0.88 (s)
19	CH_3	20.37	1.05 (s)
20	C_q	80.84	
21	CH_3	25.50	1.37 (s)
22	C_q	217.25	
23	CH_2	33.11	2.86 (H_α), 2.71 (H_β)
24	CH_2	38.12	1.71
25	C_q	70.82	
26	CH_3	29.39	1.18 (s)
27	CH_3	29.14	1.18 (s)
28	CH_3	28.59	1.06 (s)
29	CH_3	25.90	1.23 (s)
30	CH_3	19.75	1.31 (s)
31	CH	104.86	4.41 (d, *J* 7.7 Hz)
32	CH	75.54	3.15
33	CH	78.19	3.28
34	CH	71.68	3.26
35	CH	77.17	3.40
36	CH_2	69.93	3.78 (dd, J^2 11.9, J^3 5.8 Hz) 4.07 (dd, J^2 11.4 Hz)
37	CH	106.36	4.28 (d, *J* 7.7 Hz)
38	CH	75.16	3.18
39	CH	77.97	3.25
40	CH	71.63	3.32
41	CH	77.97	3.34
42	CH_2	62.76	3.67 (dd, J^2 11.8, J^3 5.2 Hz) 3.87 (dd, J^2 11.4 Hz)

[a] Internal standard, Me_4Si.

consistent with the introduction of sugar molecules at this site (δ 76.90→87.59). The C-1 and C-2 signals were assigned after comparison with the chemical shifts reported for ring A in similar structures reported by Kasai *et al.*[17] The observation of two anomeric carbon shifts (δ 104.86 and 106.36) and two signals attributable to C′-6 (δ 69.93) and C″-6 (δ 62.76) of carbohydrates indicates the presence of two sugar molecules linked in a 1–6 arrangement. Literature analogy and comparison of the ^{13}C chemical shifts with those of known compounds suggests that the sugar units are β-D-glucose.[13,17] Further, hydrolysis of **2** by an enzyme showing β-D-glucosidase activity and subsequent analysis of the hydrosylate by HPLC confirmed the presence of β-D-glucose.

The paucity of fragment ions in the CI–MS of **1** renders the technique unattractive for studying the fragmentation processes which were occurring within that molecule. The CI–MS of compound **2** was, however, very interesting. A fragment ion at *m/z* 342 (Scheme 4) represented the glycosidic moiety and confirmed **2** as a monodesmoside. Subsequent cleavages of this ion resulted in fragments at *m/z* 324 (342 − H_2O), 180 and 162, with the latter fragment ions being formed after rupture of the interglucosidic bond.

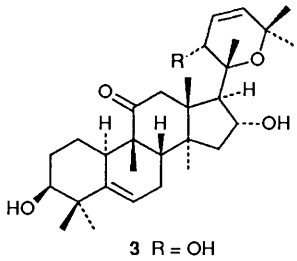
The FAB–MS of compound **2** were obtained using two



Scheme 4

different matrices (thioglycerol and formic acid–glycerol) and exhibited an $(M + H)^+$ peak at *m/z* 811. The glycosidic moiety exhibited similar fragment ions in both matrices, but there were significant differences in the cleavages of the tetracyclic framework. Matrix effects[34,35] can be important in FAB–MS and can influence which process dominates in the fragmentation of organic compounds. Thus, the peak at *m/z* 185, observed in the formic acid–glycerol matrix, was absent from the thioglycerol spectrum. Its origin may be the result of a cleavage across ring C with concomitant migration of the 30-methyl group to C-8.

FTIR spectroscopy of compound **3** suggests the presence



3 R = OH
4 R = H

of hydroxy, carbonyl and two olefinic functionalities with absorption bands at 3300–3540, 1685, 1630 and 1615 cm^{-1}, respectively. Results obtained from the APT, and the normal ^{13}C and ^{1}H NMR spectra of compound **3** are summarized in Table 2. Again using cordifolin A as the model, close similarities are observed in the substitution patterns of rings A, B, C and D. The major differences arise from substitution of the cyclised side chain. The C-22 position bears a hydroxy group that appears to be α-oriented, based on observations of NOE enhancement after irradiation at the 21-methyl site (δ 1.17). In addition, an olefinic function appears between C-23 and C-24 of compound **3**.

A very prominent peak at *m/z* 98, the base peak, was used to support the proposed structure of the cyclised side chain of compound **3**. Otherwise, **3** exhibited cleavages in the tetracyclic framework that were analogous to those observed in the fragmentations of cordifolin A **1**. For example, the fragment ion

Table 2 ^{13}C and ^{1}H NMR chemical shifts[a] of compound **3**

Carbon	Multiplicity	δ_C(ppm)	δ_H(ppm)
1	CH_2	21.68	1.29 (H_α), 1.55 (H_β)
2	CH_2	29.77	1.81 (H_α), 1.60 (H_β)
3	CH	76.90	3.42
4	C_q	42.29	
5	C_q	141.36	
6	CH	120.45	5.63 (d, *J* 5.8 Hz)
7	CH_2	24.79	1.94 (H_α), 2.38 (H_β)
8	CH	44.66	1.91
9	C_q	50.33	
10	CH	36.65	2.39
11	C_q	217.48	
12	CH_2	49.82	3.16 (H_α), 2.46 (H_β) (*J* 14.4 Hz)
13	C_q	49.15	
14	C_q	52.05	
15	CH_2	46.56	1.45 (H_α), 1.86 (H_β)
16	CH	72.68	4.65 (t, *J* 7.4 Hz)
17	CH	57.84	2.41 (d, *J* 5.5 Hz)
18	CH_3	20.30	0.88 (s)
19	CH_3	20.25	1.09 (s)
20	C_q	77.53	
21	CH_3	22.03	1.17 (s)
22	CH	78.07	4.24 (dd, *J* 0.55, 7.5 Hz)
23	CH	142.57	5.71 (dd, *J* 7.2, 15.7 Hz)
24	CH	126.71	5.92 (dd, *J* 0.66, 15.8 Hz)
25	C_q	71.24	
26	CH_3	29.88	1.27 (s)
27	CH_3	29.88	1.27 (s)
28	CH_3	28.21	1.02 (s)
29	CH_3	26.05	1.14 (s)
30	CH_3	19.56	1.30 (s)

[a] Internal standard, Me_4Si.

at *m/z* 389 ($C_{24}H_{37}O_4$) represented the tetracyclic framework which remained after the loss of the side chain. Other important fragments occurred at *m/z* 237, 173, 134 and 119. The pronounced peak in the CI–MS of **3** may be due to $(389 + NH_3)^+$ and this adduct suggests that cleavages involving that cyclised side chain occurred very early in the fragmentation process, even under CI conditions. A prominent peak at *m/z* 309 in the FAB–MS of **3** was the result of C(17)–C(2) bond rupture. Apparently, elimination of water molecules, involving both the C-3 and the C-16 hydroxy functions had previously occurred. A similar cleavage was detected among the cucurbitacins of *Hemsleya carnosiflora*,[17] but this fragmentation process was hitherto unobserved among the cucurbitacins of *Fevillea cordifolia*. The important fragments of **3** are summarized in Scheme 5.

Mass spectrometry was used to elucidate the structure of a new cucurbitacin **4** in the following manner. High resolution mass spectrometry (EI) indicated a molecular weight of 470.3403 and a molecular formula of $C_{30}H_{46}O_4$. FAB–MS, after peak matching using internal standards, confirmed the molecular ion by showing a $(M + H)^+$ peak at 471.347. The fact that the molecular ion in the EI spectrum was the base peak implied that the cleavages involving the side chain, facile processes in the other cucurbitacins studied, were not very dominant in compound **4**. This suggested that the side chain of **4** was relatively unfunctionalized. An interesting peak was observed at *m/z* 389.2519, wherein all the oxygen atoms present in the molecular formula were retained on the tetracyclic framework (Scheme 6). The prominent peak at *m/z* 319 was reminiscent of a McLafferty-type cleavage[36] involving a C-1 proton and the 11-oxo function which was reported for bryogenin[18] and which contained rings C, D and the cyclized side chain. The other cleavages of the tetracyclic framework were consistent with the presence of substituents in rings A, B, C and D that are similar in both cordifolin A and compound **4**. In fact, the difference between **3** and **4** resides in the presence of the 22-hydroxy function in **3**, whereas compound **4** appears to be a 22-deoxocucurbitacin. The occurrence of 22-deoxocucurbitacins has been previously reported,[10] but this constitutes the first indication of a 22-deoxocucurbitacin in *Fevillea cordifolia*. Sample size precluded the acquisition of supporting NMR data for **4**, thus this structure assignment is tentative.

Experimental

The procedure for isolation and purification of cordifolin A **1** from *Fevillea cordifolia*, as well as experimental details for acquisition of NMR spectra have been described previously.[21] Cucurbitacins **3** and **4** were obtained from chromatographic fractions eluting at retention times of 65.8 and 74.4 min, respectively.

The ground endosperm of seeds of *Fevillea cordifolia* (1.1 kg) was extracted overnight using methanol (2 dm^3) to afford a brown gum (61.1 g). The chloroform-soluble extract was removed and 16.3 of the residue was separated by column chromatography over silica gel (ethyl acetate–ethanol–water, 5:2:0.5), giving five fractions. Fraction 3 (3.3 g) was further purified by preparative reverse phase HPLC (C_8, 10 μm), using a stepwise gradient of methanol (A) and water as follows: 0 min (20% A); 45 min (90% A); 60 min (90% A). The fraction eluting at 19.1 min (86 mg) was finally purified over silica gel (0.25 mm, 20 × 20 cm, Merck) using double development in a mobile phase comprising chloroform–ethanol (7:3), affording **2** as the major band (28 mg).

The mass spectral conditions were as follows: MS–MS measurements were performed on a ZAB-E mass spectrometer (V.G. Analytical Ltd.) fitted with a MIKES collision gas cell at the intermediate focal plane. Samples were ionized by electron impact (70 eV) or by 8 keV Xe bombardment of samples which were previously dissolved in a thioglycerol matrix. The ions were accelerated to 8 keV and were focussed into the collision cell containing the He target gas at a pressure sufficient to attenuate the incident beam by 50%. Product ions were energy analysed and were detected using an off-axis electron multiplier, the deflector plate being held at a potential of −20 kV.

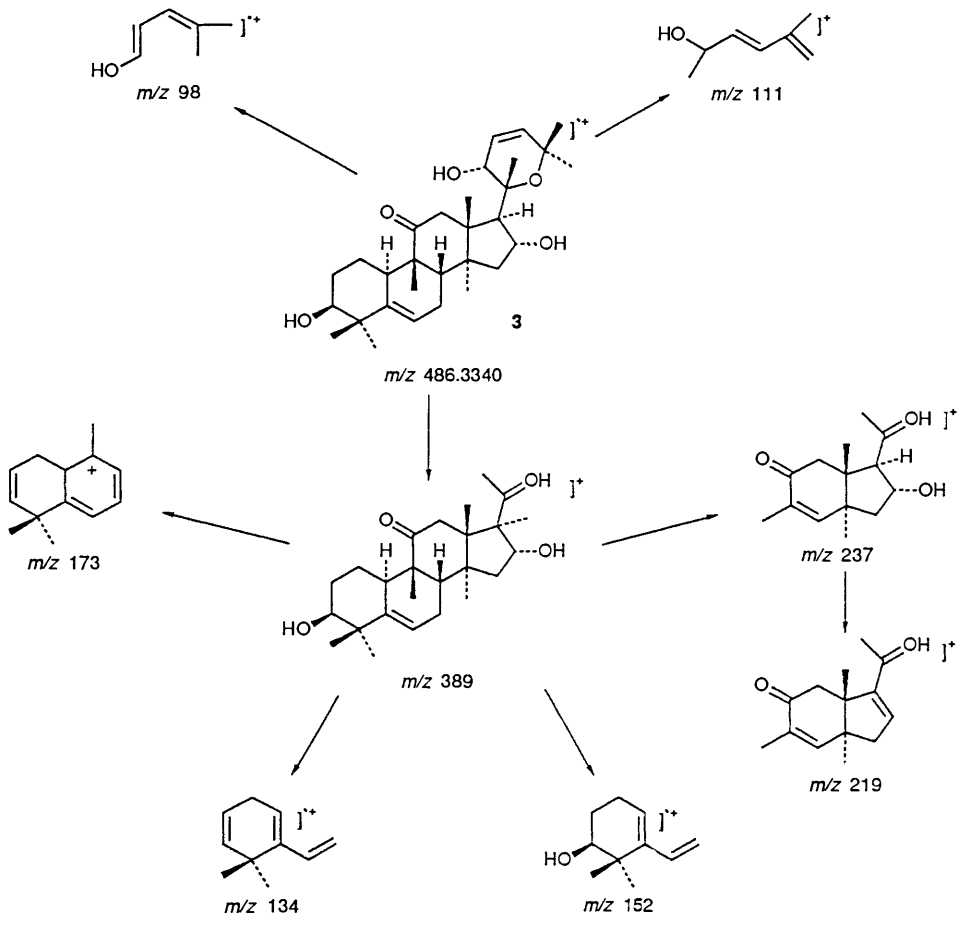
Accurate masses (±6 ppm) were determined by peak matching using secondary standards added to the matrix. Twenty such standards, authenticated by intercomparison and by reference to salt cluster and FAB matrix peaks, were used to cover the mass range 143 to 506 Da thus allowing the determination of the mass values for the compounds investigated.

Cordifolin A diglucoside **2**; m.p. 153–155 °C; ^{1}H and ^{13}C NMR data are given in Table 1; ν_{max}/cm^{-1} 3220–3590, 1685 and 1630; the highest observable EI–MS peak was at *m/z* 587.3537 ($C_{34}H_{51}O_8$); FAB–MAS shows $(M + H)^+$ at *m/z* 811.448 which, after peak matching using internal standards, gave a molecular formula of $C_{42}H_{57}O_{15}$.

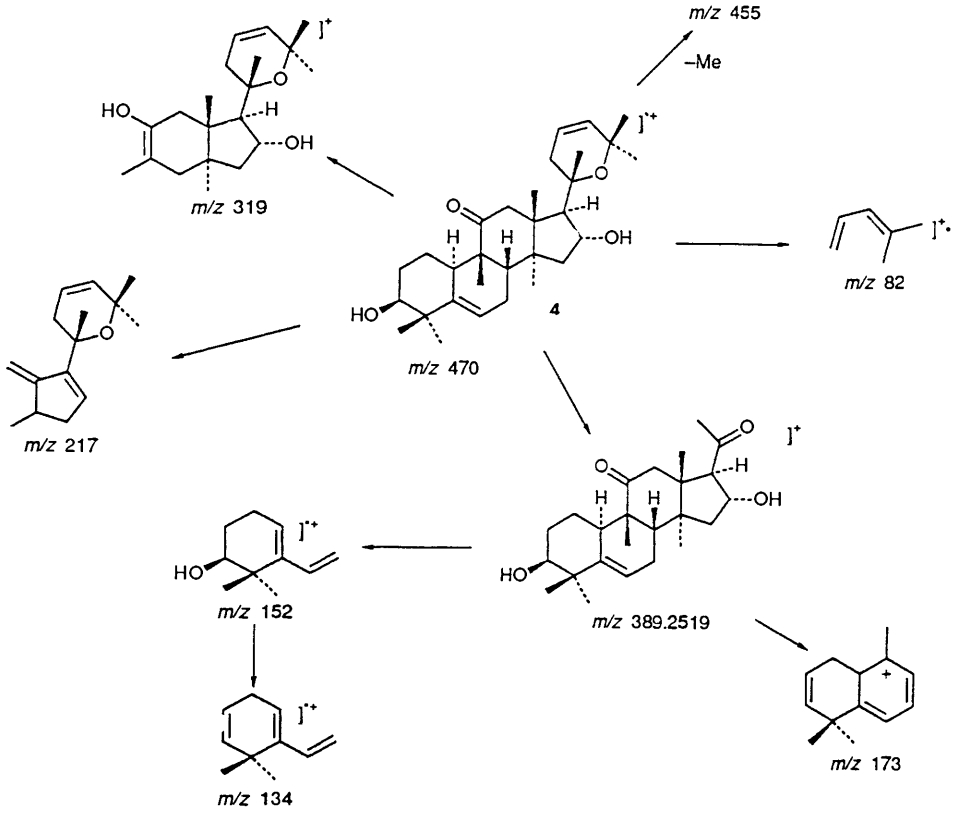
Cordifolin B **3**; m.p. 210–211 °C; ^{1}H and ^{13}C NMR data are given in Table 2; ν_{max}/cm^{-1} 3300–3540, 1685, 1630 and 1615 (Found: M^+, 486.3340. $C_{30}H_{46}O_5$ requires 486.3345). FAB–MS (glycerol) and CI–MS (ammonia) confirms M^+ by showing $(M + H)^+$, 487.

Cordifolin C **4**; m.p. 126–128 °C; ν_{max}/cm^{-1} 3440, 1680 and 1640 (Found: M^+, 470.3403. $C_{30}H_{46}O_4$ requires 470.3418). FAB–MS (thioglycerol) showed $(M + H)^+$ 471.

Enzymatic Hydrolysis of Compound **2**.—To a solution of **2** (100 μg) in water (200 mm^3) was added β-D-glucosidase (200 μg; Sigma). The mixture was incubated at 36 °C for 72 h, after which the mixture was filtered and analysed by HPLC on a Perkin-Elmer chromatograph consisting of a series 410 SDS, series 210 photo diode array detector (195 nm), a LC1-100 integrator,



Scheme 5



Scheme 6

and a six-port injection valve (Rheodyne 7125). Separations utilized a mobile phase comprised of H_2SO_4 (0.005 mol dm^{-3}) at a flow rate of 0.5 cm^3 min^{-1} and a column capable of separating sugars (Biorad HPX-87H, 300 × 7.8 mm, with guard). The sample peak was identified by comparison with an authentic sample of β-D-glucose.

Acknowledgements

One of us (L. B. N. J.) gratefully acknowledges financial support from the Scientific Research Council, Jamaica. We thank the Science and Engineering Research Council (UK) for the use of research facilities. These investigations were partially supported by the Natural Sciences and Engineering Research Council of Canada and by the Alberta Heritage Foundation for Medical Research.

References

1 J. Schmidt and S. Huneck, *Org. Mass Spectrom.*, 1979, **14**, 646.
2 J. Schmidt and S. Huneck, *Org. Mass Spectrom.*, 1979, **14**, 656.
3 P. T. Holland and A. L. Wilkin, *Org. Mass Spectrom.*, 1979, **14**, 162.
4 K. P. Madhusudanan, C. Singh and D. Fraisse, *Org. Mass Spectrom.*, 1986, **21**, 131.
5 J. R. Dias, *Org. Mass Spectrom.*, 1976, **11**, 333.
6 S. B. Mahato, K. S. Sarkar and G. Poddar, *Phytochemistry*, 1988, **27**, 3037.
7 H. D. Beckey and H. R. Schulten, *Angew. Chem.*, 1975, **14**, 403.
8 M. Barber, R. S. Bardoli, R. D. Sedgewick and A. N. Tyler, *J. Chem. Soc., Chem. Commun.*, 1981, 325.
9 H. Kambara and S. Hishida, *Org. Mass Spectrom.*, 1981, **16**, 167.
10 D. Lavie and E. Glotter, *Progress in the Chemistry of Organic Natural Products*, Springer-Verlag, New York, 1971, vol. 29, 307.
11 V. V. Velde and D. Lavie, *Tetrahedron*, 1983, **39**, 317.
12 C.-T. Che, X. Fang, C. H. Phoebe, Jr., A. D. Kinghorn, N. R. Farnsworth, B. Yelling and S. M. Hecht, *J. Nat. Prod.*, 1985, **48**, 429.
13 W. A. Laurie, D. McHale and J. B. Sheridan, *Phytochemistry*, 1985, **24**, 2659.
14 K. S. Reddy, A. J. Amonkar, T. G. McCloud, C. J. Chang and J. M. Cassady, *Phytochemistry*, 1988, **27**, 3781.
15 A. J. Amonkar, T. G. McCloud, C.-J. Chang, J. A. Scienz-Renauld and J. M. Cassady, *Phytochemistry*, 1985, **24**, 1803.
16 C. B. Gamlath, A. A. L. Gunatilaka, K. A. Alvi, A. Rahman and S. Balasubramaniam, *Phytochemistry*, 1988, **27**, 3225.
17 R. Kasai, K. Matsumoto, R. L. Nie, T. Morita, A. Awazu, J. Zhou and O. Tanaka, *Phytochemistry*, 1987, **26**, 1371.
18 H. E. Audier and B. C. Das, *Tetrahedron Lett.*, 1966, 2205.
19 S. M. Kupchan, R. M. Smith, Y. Aynechchi and M. Maruyama, *J. Org. Chem.*, 1970, **35**, 2891.
20 H. Budzikiewiez, C. Djerassi and D. H. Williams, *Structure Elucidation of Natural Products by Mass Spectrometry 2*, ch. 23, Holden-Day, San Francisco, 1964.
21 L. B. Johnson, P. B. Reese, E. V. Roberts, L. K. P. Lam and J. C. Vederas, *J. Chem. Soc., Perkin Trans. 1*, 1989, 2111, and references therein.
22 R. Muccino and C. Djerassi, *J. Am. Chem. Soc.*, 1974, **96**, 556; 1973, **95**, 8726.
23 C. B. Gamlath, A. A. L. Gunatilaka and S. Subramaniam, *J. Chem. Soc., Perkin Trans. 1*, 1989, 2259.
24 C. J. Porter, J. H. Beynon and T. Ast, *Org. Mass Spectrom.*, 1981, **16**, 101.
25 S. L. Platt and J. N. Shoolery, *J. Magn. Reson.*, 1982, **46**, 535.
26 R. A. Komoroski, E. C. Gregg, J. P. Shockcor and J. M. Geckle, *Magnetic Resonance in Chemistry*, 1986, **24**, 534.
27 T. H. Mareci and R. Freeman, *J. Magn. Reson.*, 1982, **48**, 158.
28 D. L. Turner, *J. Magn. Reson.*, 1982, **49**, 175.
29 H. Kessler, C. Griesinger, J. Zarbock and H. R. Loosli, *J. Magn. Reson.*, 1984, **57**, 331.
30 A. E. Derome, *Modern NMR Techniques for Chemistry Research*, Pergamon, Oxford, 1987.
31 J. K. M. Sanders and B. K. Hunter, *Modern NMR Spectroscopy*, Oxford University, Oxford, 1987.
32 D. Neuhaus, *J. Magn. Reson.*, 1983, **53**, 109.
33 M. Kinns and J. K. M. Sanders, *J. Magn. Reson.*, 1984, **56**, 518.
34 G. Puzo and J.-C. Prome, *Org. Mass Spectrom.*, 1984, **19**, 448.
35 Y. Tondeur, A. J. Clifford and L. M. De Luca, *Org. Mass Spectrom.*, 1985, **20**, 157.
36 A. Maccoll, *Org. Mass Spectrom.*, 1988, **23**, 381.

Paper 1/01262B
Received 18*th March* 1991
Accepted 10*th June* 1991