HETEROCYCLES, Vol. 53, No12, 2000, pp. 2689 - 2700, Received, 7th August, 2000

MASS SPECTRAL ANALYSIS OF CARDIAC STEROIDS ISOLATED FROM *ELAEODENDRON GLAUCUM*

Hiroko F. Kasai,[1*] Ken-ichi Kawai,[1] and Kazutake Shimada[2]

[1]Faculty of Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan

[2]Faculty of Pharmaceutical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-0934, Japan

Abstract — The electron ionization mass spectrometric data of three cardiac glycosides having an unusual sugar linkage, elaeodendrosides A, B and C, are reported. The decomposition patterns upon EI-MS were investigated by means of collision induced dissociation utilizing *B/E* linked scan experiments and deuterium labeling techniques. Besides the fragment ions related to aglycones, elaeodendroside A which is a 12-keto steroid, produces decomposition ions derived from cleavage of steroidal C- and D-rings. Elaeodendrosides B and C, which have a six-membered hemiacetal ring, generate ions derived from the cleavage of sugar rings and the aforementioned unusual sugar linkage.

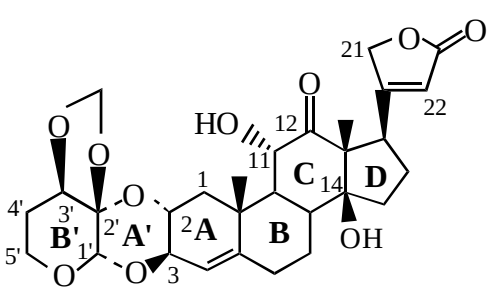
INTRODUCTION

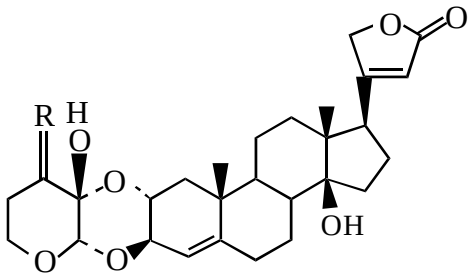
Cardiac glycosides have been used for more than 200 years to treat congestive heart failure

and other cardiac diseases. Elaeodendroside derivatives belong to the cardenolide family and were isolated from seeds of *Elaeodendron glaucum.* These glycosides are remarkable for the structure of their unusual sugar linkage (dioxane-type six-membered ring), which is not cleaved by the usual enzymatic or acid treatments. Their cardiac activities have already been reported,[1,2] but few studies have examined their MS spectroscopic properties. MS spectrometry is known to be an effective method for structure elucidation and to be suited to the study of compounds available only in limited amounts. In addition, collision induced dissociation (CID) spectra are often found to provide more informative structural data. MS spectra of cardiac glycosides provide useful structural information, as well as important and structurally diagnostic fragmentation in the case of compounds having ordinary sugar linkages that have already been uncovered.[3-5]

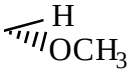
It would be interesting to investigate the pattern of decomposition of these structurally unique heterocyclic compounds having 1,4-dioxan (A'-ring) next to oxygenated tetrahydropyran (B'-ring), although MS spectral fragment patterns of 1,4-dioxan or oxygenated tetrahydropyran have already been reported.[6] The behavior of a 12-keto steroid, elaeodendroside A, is also interesting, though Djerassi *et al.* reported the fragmentations of several keto steroids.[7-9]

We report and discuss herein the MS spectra of elaeodendrosides A (**1**), B (**2**) and C (**3**) obtained under EI mode, investigating the decomposition processes of important fragments upon electron ionization mass spectrometry (EI-MS) of these compounds (**1-3**). To identify which hydrogen atom is lost or removed and identify the site of cleavage, we synthesized deuterated **1**(**1**-d_3) and **3**(**3**-d_3). Analysis of their EI-MS spectra suggested fragmentation pathways. Structures of these fragments were investigated by CID and clarified by high-resolution MS spectrometry.



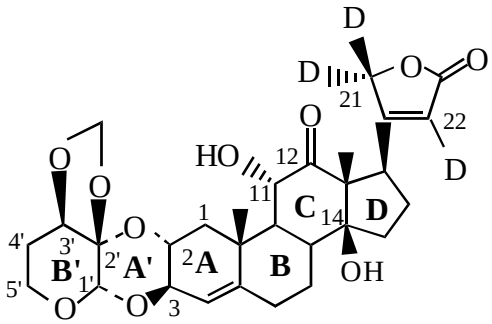


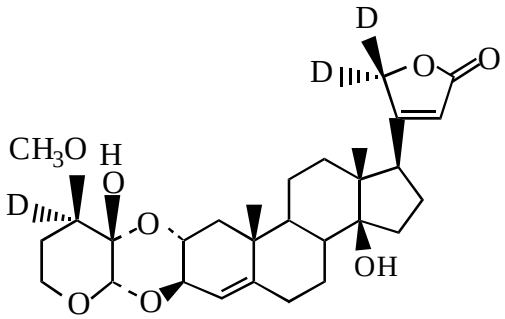
elaeodendroside A (**1**)

elaeodendroside B (**2**) (R= )

elaeodendroside C (**3**) (R= )

Figure 1. Structures of compounds (**1-3**)





21,22-trideuterated
elaeodendroside A (**1**-d $_3$)

3'α, 21-trideuterated
elaeodendroside C (**3**-d $_3$)

Figure 2. Labeled compounds

RESULTS AND DISCUSSION

It is well known that MS spectra of cardiac glycosides provide useful information on the molecular weight as well as the structure of both aglycone and sugar moieties. In the case of **1-3** (**Figure 1**) with unusual sugar linkages, the dioxane ring is expected to be an ionization center and also a fragmentation site. Important fragments obtained under EI mode are discussed in relation to possible mechanisms of their formation. The relative

abundances of significant ions (**Schemes 1-4**) in EI-MS of compounds (**1-3**) and labeled compounds (**1**-d_3 and **3**-d_3, **Figure 2**) are summarized in **Table 1**.

Table 1. *m/z* Values and relative abundances (% of base peak) of significant ions in the EI mass spectra.

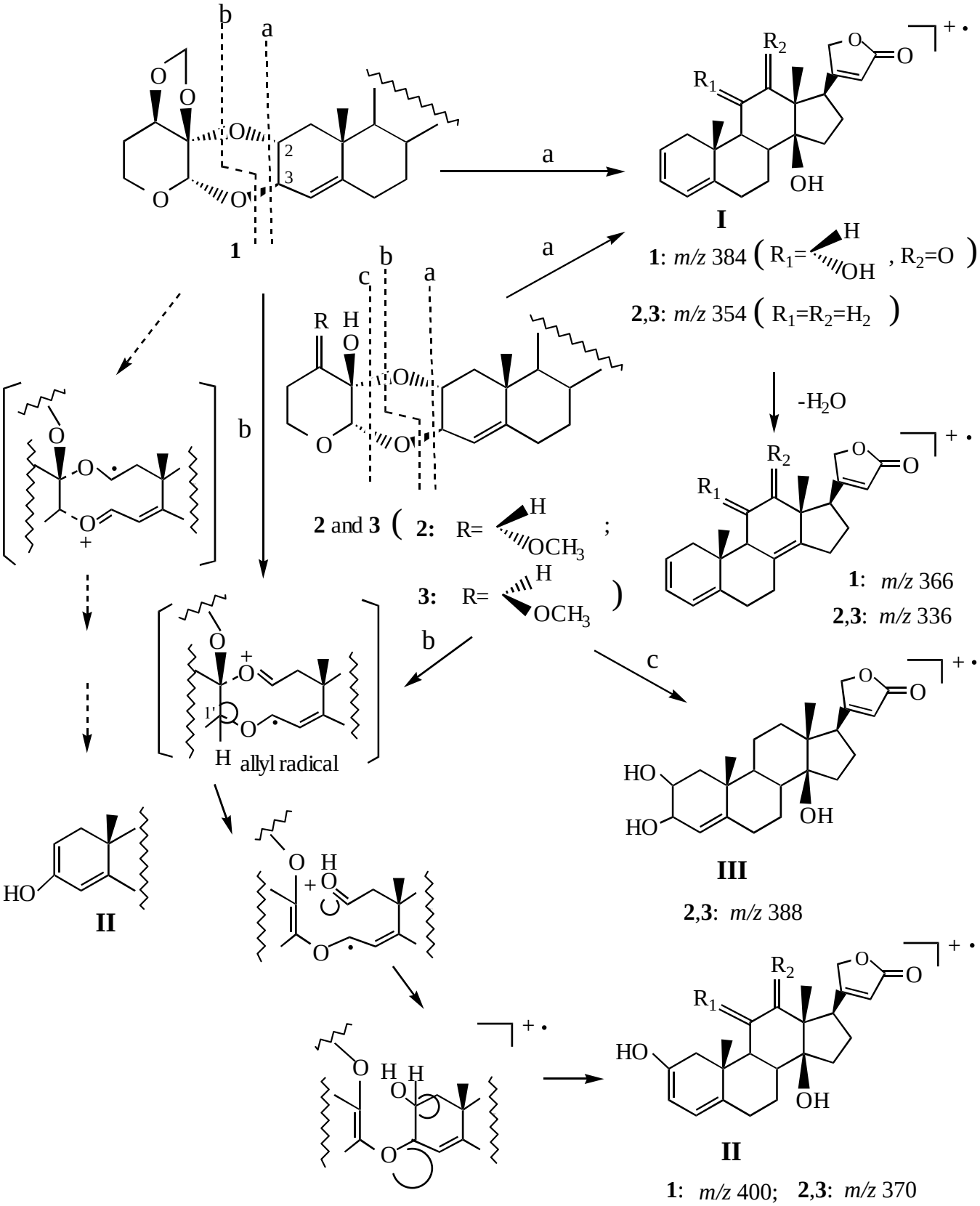
Ions	Compounds				
	1	**1-d_3**	**2**	**3**	**3-d_3**
M	544 (16)	547 (58)	516 (4)	516 (4)	519 (14)
M-H_2O	526 (2)		498 (6)	498 (28)	500 (31)
M-$2H_2O$				480 (7)	
VI				453 (47)	455 (29)
V	446 (2)	446 (32)			
VII				422 (31)	424 (48)
III			388 (13)	388 (28)	390 (50)
II	400 (4)	403 (42)	370 (93)	370 (88)	372 (98)
I	384 (11)	387 (74)	354 (100)	354 (100)	356 (100)
I-H_2O	366 (22)	369 (100)	336 (11)	336 (20)	338 (19)
I-$2H_2O$	348 (10)				
IV	181 (100)	184 (92)			

In the EI-MS spectra of the deuterated compounds, the peaks were shifted by 2 or 3 mass units from the peaks of the natural compounds (**Table 1**). Regarding compound (**1**), the peaks at *m/z* 384 (**I**) and *m/z* 400 (**II**) were shifted to *m/z* 387 and *m/z* 403, respectively, in the MS spectrum of **1**-d_3. Regarding compounds (**2**) and (**3**), the peaks at *m/z* 354 (**I**), *m/z* 370 (**II**), and *m/z* 388 (**III**) were shifted to *m/z* 356, *m/z* 372, and *m/z* 390, respectively, in the MS spectrum of **3**-d_3.

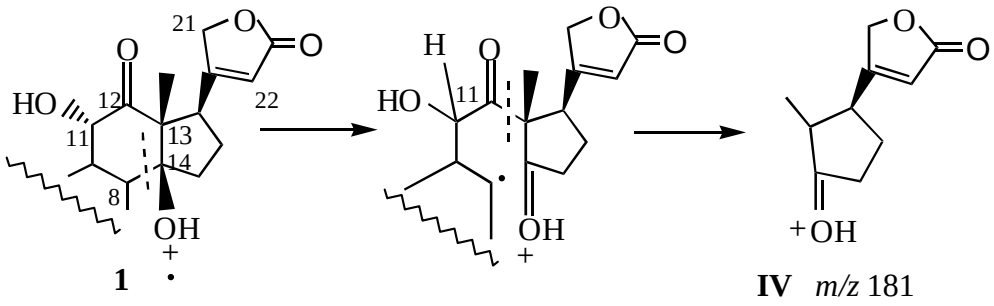
An analysis of these MS spectra suggests that decomposition occurs *via* the cleavage of the dioxane ring, as presented in **Scheme 1**, yielding ions (**I**) (route a), (**II**) (route b), and (**III**) (route c). Thus, these important primary fragmentations involve the cleavage of the dioxane ring and the release of ions related to aglycone.

Regarding ion (**II**), although a positional isomer with a hydroxyl group at either the C-2 or the C-3 position could be formed, the hydroxyl group is expected to preferentially form at the C-2 position because an allyl radical-transition state (see **Scheme 1**) for the cleavage process between the sugar and the aglycone would be more stable than the other state leading to an isomer with a hydroxyl group at the C-3 position.

Cation (**IV**), which produces a peak at *m/z* 181, is the base peak in compound (**1**), though ion (**I**) is the most abundant ion in compounds (**2**) and (**3**). In addition, ion (**V**), which produces a peak at *m/z* 446, is also observed in compound (**1**). Since neither **IV** or **V** are observed in compounds (**2**) and (**3**), the formation of **IV** and **V** is presumably influenced by the existence of a hydroxyl group at C-11 and a carbonyl group at C-12 in compound (**1**). In the EI-MS spectrum of the 21,22-trideuterated compound (**1**-d_3), the peak at *m/z* 181 (**IV**) was shifted to *m/z* 184, suggesting that hydrogens at either C-21 or C-22 are not removed during the formation of **IV**. A possible explanation for the formation of **IV** is shown in **Scheme 2**, in which the cleavage of the carbon-carbon bond (C-8 - C-14) and the subsequent hydrogen transfer from C-11 are expected to yield a stable oxonium ion (**IV**).



Scheme 1. Mechanism of the formation of **I, II** and **III**



Scheme 2. Mechanism of the formation of **IV**

Additional evidence for the presence of a five-membered unsaturated lactone and a hydroxyl group in **IV**, of *m/z* 181, was obtained by CID of this ion recorded as *B/E* linked scan experiments. As presented in **Figure 3**, the spectrum shows the main product ions at *m/z* 97 and *m/z* 163 corresponding to the lactone and loss of water from the precursor ion of *m/z* 181, respectively.

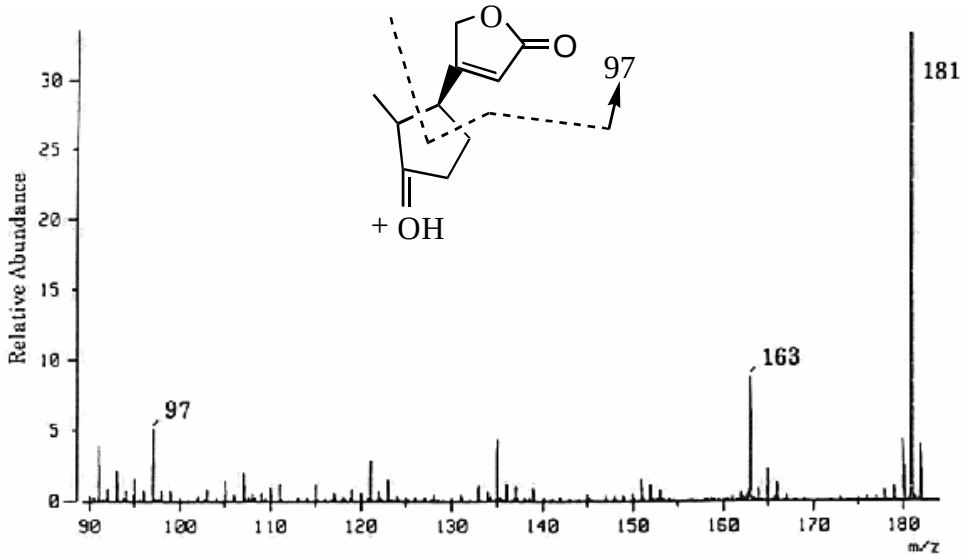
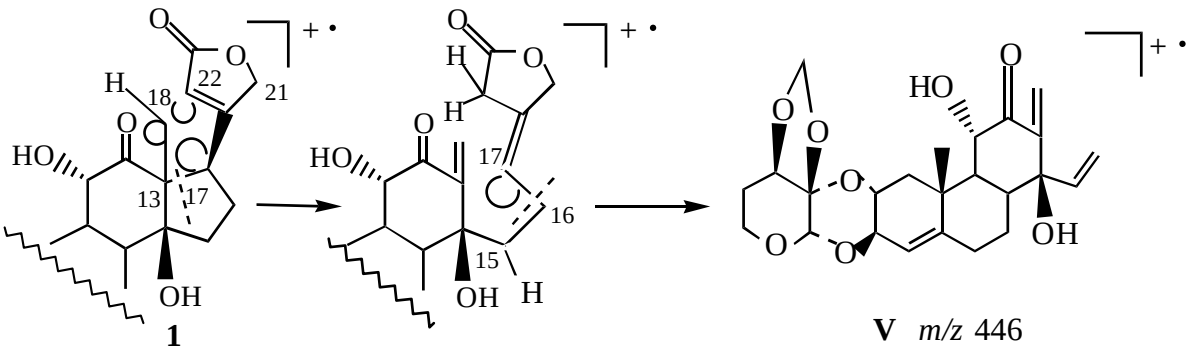


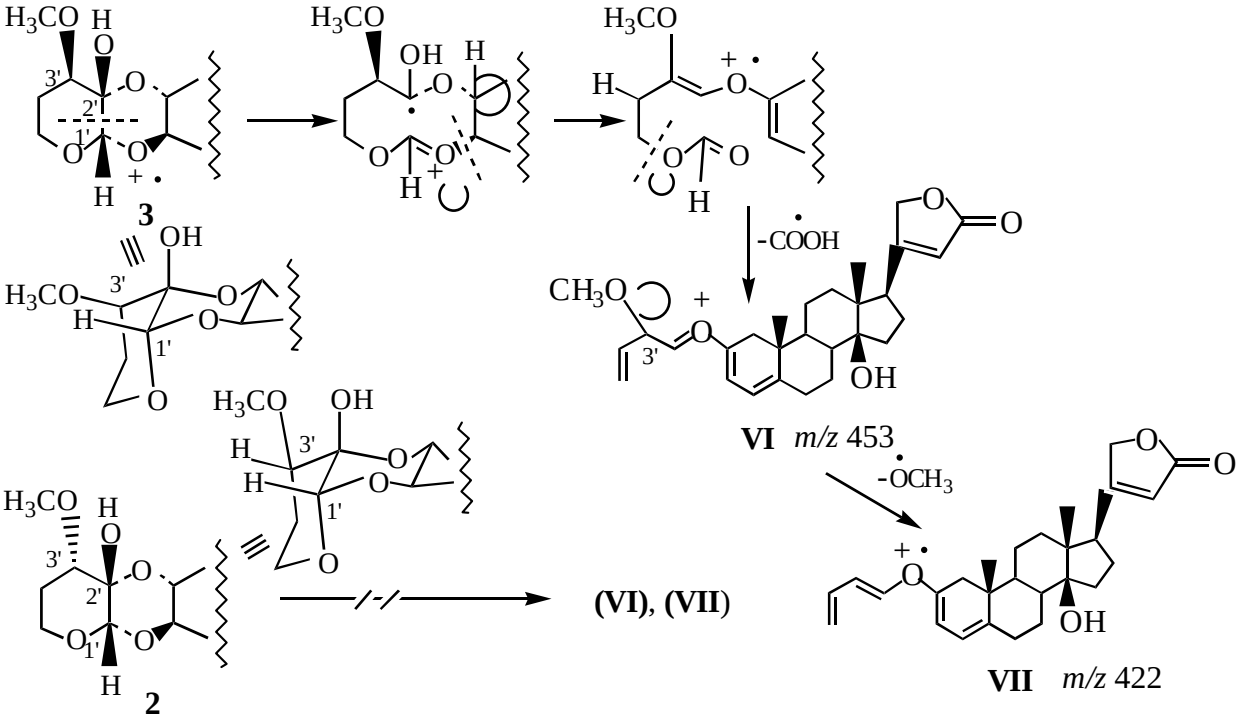
Figure 3. CID spectrum of **IV** of *m/z* 181

As to the formation of **V**, cleavage of the carbon-carbon bond (C-13-C-17) involving a McLafferty rearrangement of a hydrogen atom from the C-18 position as mentioned previously,[10,11] and additional cleavage of the C-16-C-17 bond yield ion (**V**) of *m/z* 446. In the EI-MS spectrum of 21,22-trideuterated compound (**1-**d_3), the peak at *m/z* 446 (**V**) was not shifted, supporting the mechanism outlined in **Scheme 3**.



Scheme 3. Mechanism of the formation of **V**

Ions (**VI**) at *m/z* 453 and (**VII**) at *m/z* 422 were observed in the spectrum of compound (**3**) but not in that of **2**. The stereochemistry at the C-3' position substituted a methoxyl group appears to influence these formations, although Mandelbaum reported about stereochemical effects in MS spectrometry.[12-13] In the EI mass spectrum of 3'α,21-trideuterated compound (**3-**d_3), the peak at *m/z* 500 [519$(M)^+$-HOD] indicated that dehydration occurred at the C-2' (OH) and C-3' (D) positions. As already reported concerning the cleavage of 1,4-dioxans,[6] the fragmentation appears to occur by α-cleavage of C-1' - C-2' bond of compound (**3**) to yield a highly stable oxonium ion followed by losses of the elements of carboxylic acid and subsequently a methoxyl group. Peaks at *m/z* 453 (**VI**) and *m/z* 422 (**VII**) were shifted to *m/z* 455 and *m/z* 424, respectively, in trideuterated compound (**3-**d_3), indicating that these ions involve the unsaturated lactone and loss of HOD at C-2' and C-3'. In compound (**3**), owing to the lower stability produced by the steric effect between the axial-methoxyl group at the C-3' position and the hydrogen atom at the C-1' position, consecutive cleavages are expected to progress smoothly and subsequent losses of H_2O, CO_2H, and OCH_3 extend to form **VI** and **VII**, respectively. On the contrary, in compound (**2**) whose methoxyl group at the C-3' position is equatorial, in the absence of the previously discussed configurational factor, the aforementioned subsequent reaction would not progress. One plausible mechanism for the formation of **VI** and **VII** is illustrated in **Scheme 4**.



Scheme 4. Mechanism of the formation of **VI** and **VII**

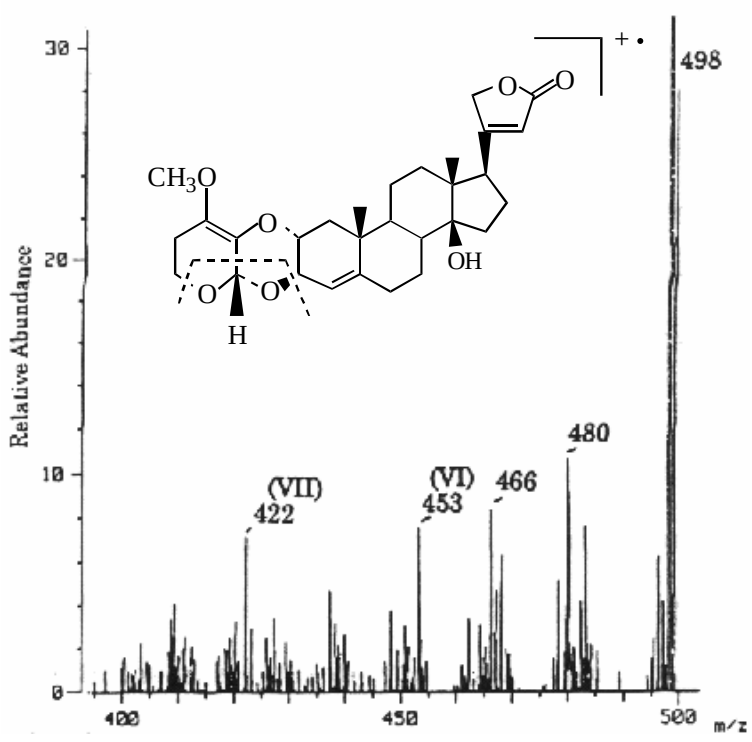


Figure 4. CID spectrum of ion of *m/z* 498

CID of the ion of *m/z* 498 of compound (**2**), recorded as *B/E* linked scan experiments, yielded fragments (**VI**) at *m/z* 453 and (**VII**) at *m/z* 422 besides the ions at *m/z*480[498-H_2O]$^+$ and *m/z*

466[498-CH_3OH]$^+$ as presented in **Figure 4**, suggesting structural information.

The presence of functional groups or different stabilities, due to differing stereochemistry, influences whether or not fragmentation proceeds.

EXPERIMENTAL

Plant material — Elaeodendrosides A (**1**), B (**2**) and C (**3**) were previously isolated from seeds of *elaeodendron glaucum* collected in India, and characterized.[14-15]

Instrumentation — The EI mode MS were performed with a JEOL JMS-600W double-focusing (EB geometry) mass spectrometer with a JEOL MS-MP09 data system (Tokyo, Japan) which was operated with a filament current of 300 μA, accelerating voltage of 3 kV, an electron energy of 70 V, and an ion chamber temperature of 200℃. The samples were introduced using the direct insertion technique at a probe temperature of 60-300℃ and a rate of 128℃/min. The resolutions for low- and high-resolution MS were 500 and 3000, respectively, and perfluorokerosene was used as a standard. CID spectra were obtained with a JEOL JMS-SX102 double-focusing (BE geometry) mass spectrometer with a JEOL complement data system (Tokyo, Japan) which was operated with an accelerating voltage of 10 kV. A *B/E* constant linked scan was generated using sufficient helium as the collision gas to reduce the abundance of the standard beam intensity by 30 %.

^{1}H NMR (500 MHz) spectra were recorded on JEOL JNM-LA500 from a $CDCl_3$ solution of the product using TMS as an internal standard. Deuterium solvents and reagents were research grade products from Isotec inc. (Miamisburg, Oh, U.S.A.) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan), respectively, and were used without further purification.

The deuterium isotopomers were synthesized by treatment with CD_3OD (99.8 atom % D) - D_2O (99.9 atom %D) (10:1) in the presence of sodium carbonate. Compound (**2**) was transformed into 21, 3'α-trideuterated compound (**3**-d_3) (MW: 519) as reported previously.[15] Treating compound (**1**) by the same method as in the case of compound (**2**) produced 21,22-

trideuterated compound (MW: 547, **1**-d_3). The ^{1}H-NMR spectrum of deuterated compound displayed the loss of signals at δ 6.00 (1H, br s, C22-H), δ 4.90 (1H, dd, J=1.2, 18.0 Hz,C21-H) and δ 4.78 (1H, dd, J=1.8, 18.0 Hz, C21-H) which were present in compound (**1**),[14,16] revealing possible information about the deuterated positions at C-21 and C-22. The degrees of labeling estimated by the MS spectra were better than 99.9 %.

21,22-Trideuterated elaeodendroside A (1-d_3) : Dry Na_2CO_3 (1.2 mg, 0.0037 mmol) was added to a solution of compound (**1**) (2 mg, 0.0110 mmol) in CD_3OD-D_2O (10:1; 2 mL). The solution was then stirred at 60-70 ℃ for 1 h. The reaction mixture was extracted with ethyl acetate, and the organic layer was washed with distilled water and dried over dry Na_2SO_4. After removal of the solvent, the residue was purified by silica gel column chromatography using a mixture of *n*-hexane and ethyl acetate (1:1) as an eluent to give **1**-d_3 (1.5 mg, 74.1 %) as colorless plates.

High-resolution MS spectrometry — Ion (**I**): *m/z* 384.1958 (Calculated for $C_{23}H_{28}O_5$: 384.1937)(for **1**); *m/z* 354.2207 (Calculated for $C_{23}H_{30}O_3$: 354.2195)(for **2**). Ion [**I**-H_2O]: *m/z* 366.1818 (Calculated for $C_{23}H_{26}O_4$: 366.1831) (for **1**); *m/z* 336.2062 (Calculated for $C_{23}H_{28}O_2$: 336.2089) (for **2**). Ion [**I**-2H_2O]: *m/z* 348.1729 (Calculated for $C_{23}H_{24}O_3$: 348.1725) (for **1**). Ion (**II**): *m/z* 400.1886 (Calculated for $C_{23}H_{28}O_6$: 400.1886)(for **1**);*m/z* 370.2131 (Calculated for $C_{23}H_{30}O_4$: 370.2144)(for **2**). Ion (**III**): *m/z* 388.2234 (Calculated for $C_{23}H_{32}O_5$: 388.2249) (for **2**). Ion(**IV**): *m/z* 181.0877 (Calculated for $C_{10}H_{13}O_3$: 181.0865). Ion (**V**): *m/z* 446.1943 (Calculated for $C_{24}H_{30}O_8$: 446.1940). Ion (**VI**): *m/z* 453.2644 (Calculated for $C_{28}H_{37}O_5$: 453.2641). Ion(**VII**): *m/z* 422.2447 (Calculated for $C_{27}H_{34}O_4$: 422.2457).

ACKNOWLEDGEMENTS

We are grateful to President T. Nambara, Assistant Prof. M. Tsubuki, and the staff of the

laboratory of organic chemistry of Hoshi University (Tokyo, Japan), for encouragement, advise, and aid in this research, respectively.

REFERENCES

1. K. Shimada, T. Kyuno, T. Nambara, and I. Uchida, *Chem. Pharm. Bull.*, 1982, **11**, 4075.
2. K. Shimada, N. Ishii, K. Ohishi, J. S. Ro, and T. Nambara, *J. Pharmacobio-Dyn.*, 1986, **9**, 755.
3. P. Brown, F. Brüschweiler, and G. R. Pettit, *Org. Mass Spectrom.*, 1971, **5**, 573.
4. F. C. Falkner, J. Frölich, and J. T. Watson, *Org. Mass Spectrom.*, 1973, **7**, 141.
5. R. Isobe, T. Komori, F. Abe, and T. Yamauchi, *Biomed. Mass Spectrom.*, 1986, **13**, 585.
6. Q. N. Porter, 'Mass Spectrometry of Heterocyclic Compounds,' 2nd. ed., John Wiley & Sons, New York, 1985, pp. 94-95, 335-339.
7. C. Djerassi, G. von Mutzenbecher, J. Fajkos, D. H. Williams, and H. Budzikiewicz, *J. Am. Chem. Soc.*, 1965, **87**, 817.
8. C. Djerassi, R. H. Shapiro, and M. Vandewalle, *J. Am. Chem. Soc.*, 1965, **87**, 4892.
9. C. Djerassi and L. Tökés, *J. Am. Chem. Soc.*, 1966, **88**, 536.
10. P. Brown, Y. Kamano, and G. R. Pettit, *Org. Mass Spectrom.*, 1972, **6**, 613.
11. M. B. E. Fayez and S. A. R. Negm, *J. Pharm. Sci.*, 1972, **61**, 765.
12. A. Mandelbaum, *Mass Spectrom. Rev.*, 1983, **2**, 223.
13. A. Mandelbaum, *Spectros. Int. J.*, 1984, **3**, 370.
14. S. M. Kupchan, I. Uchida, K. Shimada, B. Y. Fei, D. M. Stevens, A. T. Sneden, R. W. Miller, and R. F. Bryan, *J. Chem. Soc., Chem. Comm.*, **1977**, 255.
15. K. Shimada, T. Kyuno, T. Nambara, and I. Uchida, *Phytochemistry* ,1985, **24**, 1345.
16. R. Hintsche, R. Megges, D. Pfeiffer, H. J. Portius, W. Schönfeld, and K. R. H. Repke, *Eur. J. Med. Chem. –Chim. Ther.*, 1985, **20**, 9.