Mass Spectra of Some Stilbene Oligomers from *Carex* Species

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It is generally known that specimens of *Carex* species plants are resistant to insects and contain a variety of stilbene compounds. Some stilbene oligomers obtained from four kinds of *Carex* plants were analyzed by mass spectrometry. The electron impact mass spectra, secondary ionization mass spectra, and B/E linked scan spectra were measured to investigate their fragmentations.

1. Introduction

It is generally known that specimens of *Carex* species plants are resistant to insects for long periods of time. This suggests that some active ingredients might be contained in the plants. We performed structure analyses of the components which were obtained by a conventional method after methanol extraction of four kinds of *Carex* plants whose constituents had not been reported yet; *Carex foliosissima*, *Carex morrowii*, *Carex multifolia*, and *Carex ciliato-marginata*. Three kinds of stilbene compounds were isolated as constituents showing antibacterial activity. Mass spectra of these compounds as well as several kinds of derivatives obtained through methylation and acetylation of their phenolic hydroxyl groups were measured for structural determinations. With the help of nuclear magnetic resonance spectral analyses and other instrumental analyses, the three kinds of compounds were identified as (+)- α -viniferin (1), pallidol (2), and kobophenol A (3), which have been isolated from other plants and whose structures have been elucidated to be stilbene oligomers based on resveratol (3,5,4'-trihydroxystilbene) (Fig. 1). The electron impact mass spectra (EIMS) and secondary ionization mass spectra (SIMS) of stilbene oligomers were compared with each other and were investigated in terms of efficiency in determining structures of compounds and in searching for unknown compounds. In addition, their fragmentation pathways were investigated through additional B/E linked scan spectra as they were needed.

2. Experimental

2.1 Sample preparation

Four kinds of *Carex* plants were collected and immersed in methanol. And then the solvent was evaporated to obtain methanol extracts. These extracts were fractionated

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Fig. 1. Molecular structures of stilbene oligomers: (+)- α -viniferin (1), pallidol (2), and kobophenol A (3).

into hexane-, ethyl acetate-, butanol-, and water-soluble fractions. The hexane-soluble extract was further chromatographed through silica gel column. From a solvent (CHCl_3 ; CH_3OH , 10 : 1 to 10 : 4) soluble sub-fraction, (+)- α -viniferin (1) ($[\alpha]_D + 24.26^\circ$),¹⁾ pallidol (2) ($[\alpha]_D - 84.26^\circ$),²⁾ and kobophenol A (3) ($[\alpha]_D + 193.4^\circ$, mp 230–235°C)³⁾ were isolated and identified, respectively.

2.2 Spectral measurement

EIMS and SIMS spectra were obtained with a Hitachi M-2500 Mass Spectrometer and a Hitachi M-0301 Data Processing system. Application parameters for EIMS were as follows: ionization energy; 70 eV, accelerating voltage; 4 kV, and ionization chamber temperature; 220°C. Application parameters for SIMS were as follows: primary ion; Xe^+ , accelerating voltage; 9 kV, secondary ion accelerating voltage; 4 kV, and matrix; glycerol.

3. Results and Discussion

3.1 (+)- α -Viniferin (1)

The molecular ion of (+)- α -viniferin (1) which was obtained from *Carex morrowii* was not found in the EIMS spectra. However, its hexamethyl ether (4) and hexaacetate clearly showed their molecular ion peaks, m/z 762 (M^{++}) and m/z 930 (M^{+}), respectively. In the SIMS spectra of (1), a protonated $[\text{M} + \text{H}]^+$ ion (m/z 679) and a deprotonated $[\text{M} - \text{H}]^-$ ion (m/z 677) were readily observed, the negative ion peak being more identifiable. The molecular formula of (1) was determined to be $\text{C}_{42}\text{H}_{30}\text{O}_9$ by measurement of the positive ion high resolution SIMS spectrum (found: 679.1946; calcd for $\text{C}_{42}\text{H}_{31}\text{O}_9$: 679.1966).

Since the SIMS spectra of (1) and (4) contained fragment ions suggesting the structural informations, the B/E linked scan spectra were also measured (Figs. 2 and 3). The spectral analyses revealed that both of the compounds showed similar fragmenta-

Fig. 2. Linked scan SIMS (positive ion) spectrum of (+)- α -viniferin (**1**, m/z 679 $[M+H]^+$).

Fig. 3. Linked scan SIMS (positive ion) spectrum of (+)- α -viniferin hexamethyl ether (**4**, m/z 763 $[M+H]^+$).

Fig. 4. SIMS spectral fragmentations of (+)- α -viniferin (**1**, $R=H$) and (+)- α -viniferin hexamethyl ether (**4**, $R=CH_3$).

tions as shown in Fig. 4, where fragment ions m/z 345, 453, and 571 from the protonated molecular $[M+H]^+$ ion (m/z 679) in the spectrum of (**1**) shifted to those m/z 387, 509, and 641 from $[M+H]^+$ ion (m/z 763) in the spectrum of (**4**).

3.2 Pallidol (**2**)

In the EIMS spectra of pallidol (**2**), the molecular ion (m/z 454) was observed. Also, in the SIMS spectra of (**2**), the protonated molecular $[M+H]^+$ ion (m/z 455) (Fig. 5) was obtained. No significant fragment ions suggesting the molecular skeleton of (**2**) were shown in both the spectra. However, the B/E linked scan spectrum of the protonated molecular $[M+H]^+$ ion (m/z 455) in the SIMS produced daughter ions of m/z 349 and m/z 361 (Fig. 6). These daughter ions indicate elimination of m/z 94 ($C_6H_6O^+$) and m/z 106 ($C_7H_6O^+$) from the protonated molecular $[M+H]^+$ ion, being useful for partial structure analyses of (**2**) and other related compounds (Fig. 7). The same was said of the

Fig. 5. SIMS(positive ion) spectrum of pallidol (2).

Fig. 6. Linked scan SIMS(positive ion) spectrum of pallidol (2, m/z 455 $[M+H]^+$).

Fig. 7. SIMS spectral fragmentations of pallidol (2, $R=H$) and pallidol hexamethyl ether (5, $R=CH_3$).

EIMS and SIMS spectra of its hexamethyl ether (5). On the other hand, in the EIMS spectra of hexaacetyl pallidol, the molecular ion was not observed, but the $[M+H]^+$ ion (m/z 707) was observed in the SIMS spectra.

3.3 Kobophenol A (3)

The EIMS spectra of both kobophenol A (3) and its decamethyl ether (6) did not show the molecular ions, but the positive ion SIMS spectra of them clearly showed the protonated molecular $[M+H]^+$ ions of m/z 925 (Fig. 8) and m/z 1065 (Fig. 9a), respectively. And SIMS and EIMS spectra of (6) were very similar to each other, showing common ions such as m/z 928, 779, 658, and 523, which suggested a similar fragmentation pattern in both the spectra (Fig. 9a and 9b). In addition, the high resolution mass spectra (HR-EIMS) of those fragment ions of m/z 107, 121, 135, 270, and 286 in the EIMS spectra showed them to be $[CH_3OC_6H_4]^+$, $[CH_3OC_6H_4CH_2]^+$, $[CH_3OC_6H_4CO]^+$, $[CH_3OC_6H_4CHCHC_6H_3(OCH_3)_2]^+$, and $[CH_3OC_6H_4COCH_2C_6H_3(OCH_3)_2]^+$, respectively, suggesting a stepwise fragmentation (Fig. 10). The B/E linked scan spectra produced similar ions for both (3) and (6); fragment ions m/z 955, 928, and 779 were from the protonated molecular $[M+H]^+$ ion (m/z 1065) in the spectra of (6).

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Fig. 8. SIMS (positive ion) spectrum of kobophenol A (3).

Fig. 9. SIMS (positive ion) (a) and EIMS (b) spectra of kobophenol A decamethyl ether (6).

Fig. 10. Presumed formulae of basic fragment ions.

Many common features were observed among the EIMS, SIMS, and B/E linked scan spectra of (6). Therefore, B/E linked scan spectra (EIMS) of major fragment ions were measured to investigate their fragmentations (Fig. 11). The result showed that the fragmentations were clear and systematic (Figs. 12 and 13).

4. Conclusion

General findings on stilbene oligomers in this paper are as follows:

1. SIMS spectra are more useful than EIMS spectra in observing molecular ions. In addition, with SIMS spectra, negative ion spectra are more effective than positive ion

Fig. 11. Linked scan EIMS spectrum of kobophenol A decamethyl ether [$6, m/z$ 1064(M^+)].

Fig. 12. Linked scan EIMS spectral data of kobophenol A decamethyl ether [$6, m/z$ 1064(M^+)].

Fig. 13. EIMS spectral fragmentation of kobophenol A decamethyl ether (6).

spectra in detecting molecular ions.

2. Although molecular ions are hardly observable on EIMS, fragment ions of m/z 94 were readily observed, which reflects phenolic hydroxyl groups.

These findings are useful for structural analysis of unknown stilbene oligomers, and applied to structure determination of related unknown compounds.⁴⁾

References

- 1) S. Kitanaka, T. Ikezawa, K. Yasukawa, S. Yamanouchi, M. Takido, H. K. Sung, and I. H. Kim, *Chem. Pharm. Bull.*, **38**, 432 (1990).
- 2) M. A. Khan, S. G. Nabi, S. Prakash, and A. Zaman, *Phytochemistry*, **25**, 1945 (1986).
- 3) J. Kawabata, S. Ichikawa, H. Kurihara, and J. Mizutani, *Tetrahedron Lett.*, **30**, 3785 (1989); H. Kurihara, J. Kawabata, S. Ichikawa, M. Mishima, and J. Mizutani, *Phytochemistry*, **30**, 649 (1991).
- 4) H. Nozaki, M. Nakayama, and *et al.*, unpublished data.

Keywords

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