HYDROGENATION OF QUINONE COMPOUNDS DURING SECONDARY ION MASS SPECTRA MEASUREMENT

YUKIKO ISHIHARA, KUNIKATSU SHIRAHATA and HIROSHI SANO*

Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd.,
Machida-shi, Tokyo, Japan

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Antibiotics containing a quinone group show characteristic reduced pseudo-molecular ions $(M+2)^+$ and $(M+3)^+$ during the measurements of secondary ion mass spectra using glycerol as a matrix. The ratios of peak intensities $(M+2)^+$ and $(M+3)^+$ over $(M+1)^+$ increase with time. As this phenomenon is not found using sulfolane as a matrix, the quinone group seems to be hydrogenated to a hydroquinone by active hydrogen which is produced from a free hydroxyl group of the glycerol by bombardment with the Xe^+ beam. This hydrogenation reaction is specific for the quinone group.

Secondary ion (SI)-MS is an excellent ionization method for unstable natural compounds. Among the antibiotics, there are many compounds containing a quinone group, such as mitomycins A, B, C, rifamycin S, kidamycin, doxorubicin, daunorubicin, herbimycin and geldanamycin. The quinone groups are thought to play an important role in their biological activities.[1,2] During the SI-MS measurement of these compounds, it was found that the quinone group was hydrogenated to form $(M+3)^+$ ions. This hydrogenation reaction was found in all the compounds so far examined and is believed to occur generally in all compounds having a quinone group. Similar hydrogenation of quinones in mass spectrometry has been reported in the fast atom bombardment (FAB)-MS of saframycins,[3,4] the field desorption (FD) and FAB-MS of fredericamycin A,[5] and the electron impact (EI)-MS of plastoquinones.[6]

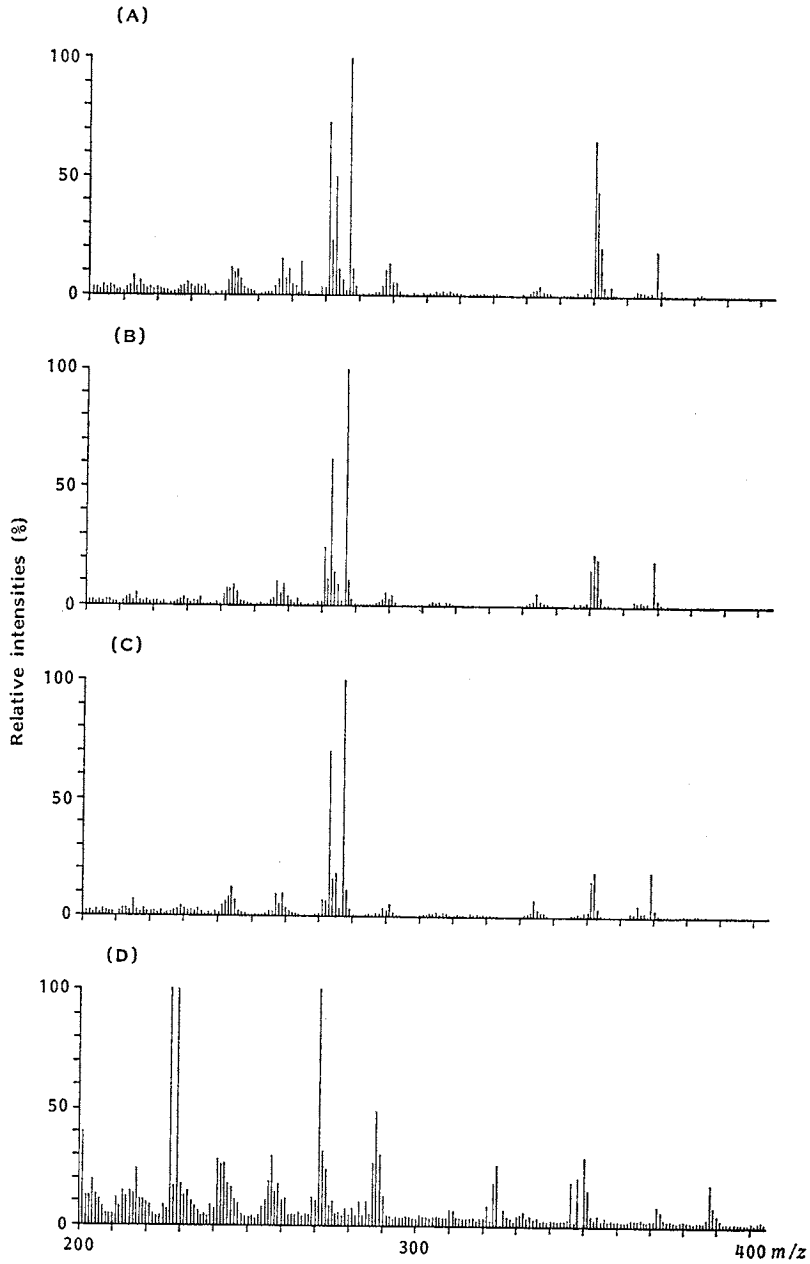
Experimental

SI-MS were recorded on a Hitachi M-80B double focusing mass spectrometer fitted with a high field magnet. The standard Hitachi M-8082 SI-MS ion source was employed to generate an 8 kV xenon ion beam. All mass spectrometric analyses were performed with a 3-kV accelerating voltage. Samples (25 μg) were dissolved in 1 μl of the following solvents: Methanol for mitomycins A, B, kidamycin and herbimycin; a 4:1 mixture of methanol and dimethyl sulfoxide for mitomycin C and a 1:1 mixture of methanol and dimethyl sulfoxide for rifamycin S, doxorubicin and daunorubicin. The solutions were mixed with glycerol (1~2 μl) on a silver target plate (2 mm wide). When sulfolane was used as a matrix, samples (50 μg) were dissolved in sulfolane (5 μl) alone on a broad silver target plate (4 mm wide). For the purpose of examing the time-course under the operating conditions of the mass spectrometer and data processor, the data acquisition was started simultaneously with xenon ion acceleration.

Results and Discussion

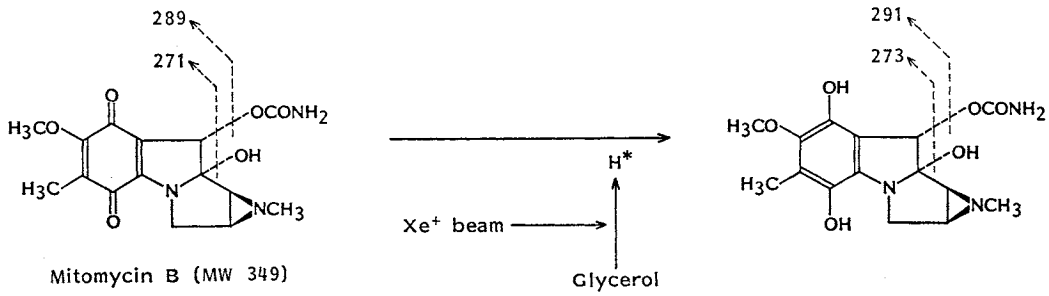
SI-MS of mitomycin B (**1**) using glycerol as a matrix are shown in Figs. 1A~1C. At the early stage of Xe^+ beam bombardment, the $(M+1)^+$ ion (m/z 350) was observed as the most intense of the pseudo-molecular ion peaks (Fig. 1A). The relative intensity of $(M+2)^+$ (m/z 351) and $(M+3)^+$ (m/z 352) increased gradually with time (Fig. 1B), and finally $(M+3)^+$ became the most intense peak

Fig. 1. SI-MS of mitomycin B at (A) 1.6 minutes, (B) 4.3 minutes and (C) 8.2 minutes after bombardment with the Xe^+ beam started, using glycerol as a matrix, and (D) at 4.3 minutes, using a sulfolane matrix.



of the three (Fig. 1C). Fragment ions (*m/z* 271 and 289) containing the quinone moiety (Fig. 2) were also observed to shift 2 mass units higher (*m/z* 273 and 291) with the change of the relative intensities of the pseudo-molecular ions. After 8 minutes, the $(M+1)^+$ ion disappeared and the conversion of $(M+1)^+$ to $(M+2)^+$ and $(M+3)^+$ was almost complete as shown in Fig. 1C, although the intensities of the last two also became weak. At this stage, the characteristic purple color for the quinone group

Fig. 2. The hypothesis about hydrogenation of mitomycin B to a hydroquinone during SI-MS measurement and diagnostic mass fragmentations.



of **1** had disappeared in the matrix on the target plate.

On the other hand, a similar SI-MS measurement was performed using sulfolane as the matrix. As sulfolane is more volatile than glycerol, a large amount of the matrix solution was put on a broad silver target plate. In this case, neither pseudo-molecular ions nor fragment ions were reduced and the purple color of mitomycin B was maintained in the remaining sulfolane solution even after 4.3 minutes (Fig. 1D). On the basis of these observations it appears that an activated hydrogen species, for instance hydrogen radicals, arise from the free hydroxyl groups of glycerol by bombardment with the Xe^+ beam and reduce the quinone moiety of mitomycin B to a hydroquinone during the SI-MS measurement (Fig. 2).

The time course of relative peak intensities for $(M+1)^+$, $(M+2)^+$ and $(M+3)^+$ using a glycerol matrix is shown in Fig. 3. Each absolute intensity of pseudo-molecular ions, $(M+1)^+$, $(M+2)^+$ and $(M+3)^+$, was averaged for 30 seconds (3 scan times) for rifamycin S (**4**) and daunorubicin (**7**) and 48 seconds (5 scan times) for the others, and expressed as a percentage of the three ions. The accuracy of the relative intensities depends on the absolute intensities of the pseudo-molecular ions. The absolute intensities of the pseudo-molecular ions and the tendency of their decay during the measurement were significantly different from one antibiotic to another. Therefore, as an indication of the accuracy, the ratio of the sum of the absolute pseudo-molecular ion intensities to that of mitomycin A measured at 0.4 minute (which was the most intense among the compounds so far examined) is shown in the figure.

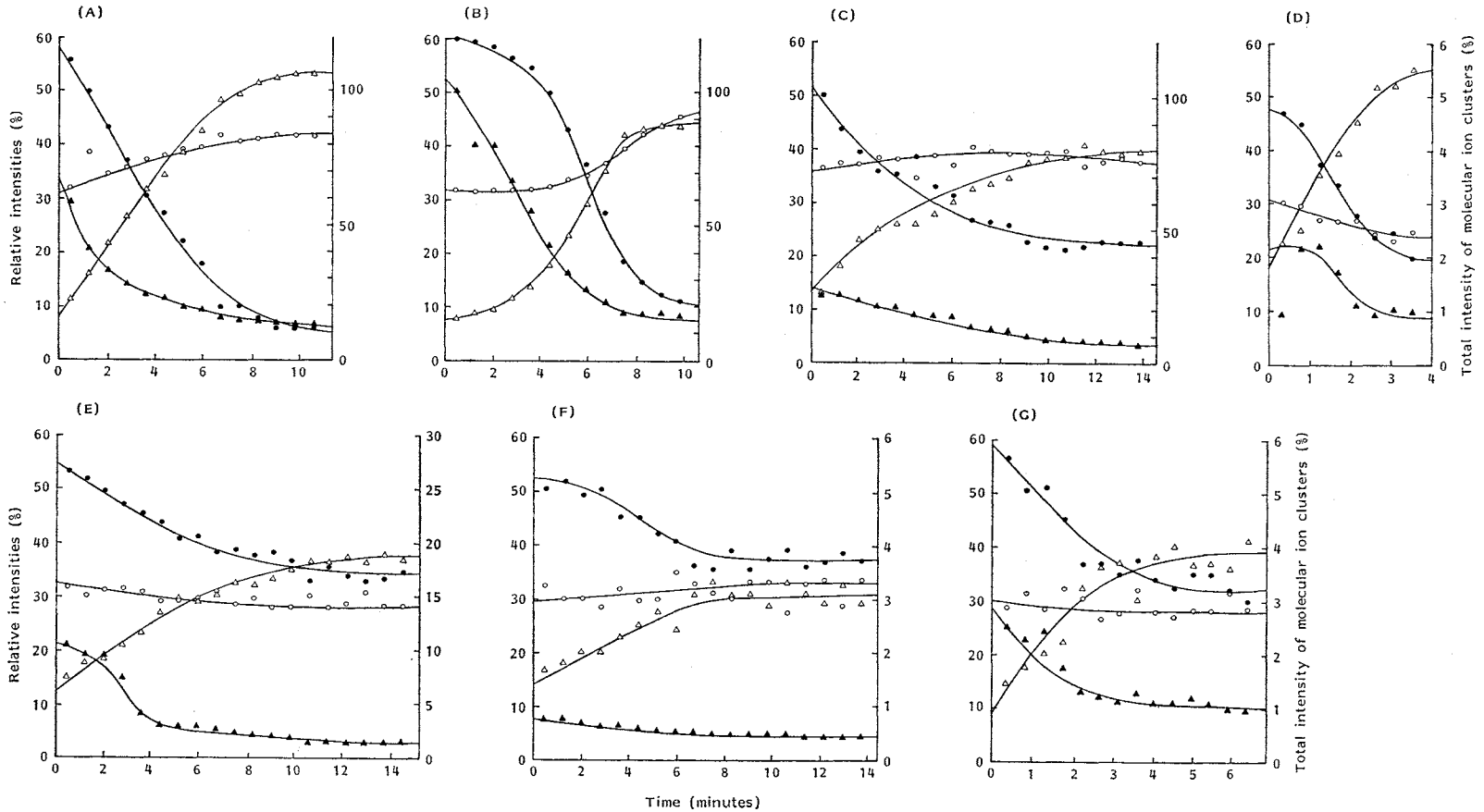
Fig. 3A well demonstrates the hydrogenation process of mitomycin B. Similar hydrogenation reactions were also observed for the other antibiotics containing a quinone moiety. In the spectra of mitomycin A (**2**), mitomycin C (**3**), rifamycin S (**4**), kidamycin (**5**), doxorubicin (**6**), daunorubicin (**7**), herbimycin (**8**) and geldanamycin (**9**), $(M+3)^+$ ions were observed as the most intense peak in the molecular ion clusters. Mitomycins (**2** and **3**), naphthoquinone (**4**) and anthraquinone antibiotics (**5**) showed a similar reaction pattern to that of **1**, and again the hydrogenation reaction was complete within 8 minutes (Fig. 3B through Fig. 3E). Although **6** and **7** are anthraquinone compounds similar to **5** and showed a similar hydrogenation pattern, the total intensities of the molecular ion clusters were weak and decreased so rapidly through the measurement that the process of hydrogenation could not be found clearly (Fig. 3F and 3G). On the contrary, **8** and **9** showed the $(M+3)^+$ peak as the most intense peak in the molecular ion clusters at an early stage of the measurement (0.4 minute).

In the case of antibiotics containing two quinone groups, such as 7-*N*,7′-*N*-dithiobistrimethyl-

Fig. 3. The time course of the relative intensities of pseudo-molecular ions and the total intensity of the molecular ion clusters.

Relative intensities of ● $(M+1)^+$, ○ $(M+2)^+$, △ $(M+3)^+$ and total intensity ▲ of molecular ion clusters.

(A) Mitomycin B (1), (B) mitomycin A (2), (C) mitomycin C (3), (D) rifamycin S (4), (E) kidamycin (5), (F) doxorubicin (6), (G) daunorubicin (7).



The total intensity is presented as the ratio of the sum of absolute pseudo-molecular ion intensities to that of mitomycin A measured at 0.4 minute.

enedimitomycins C and D,[7] more complex molecular ion clusters composed of $(M+2)^+$, $(M+3)^+$ and $(M+4)^+$ were observed. $(M+5)^+$ ions were also observed depending on the compound.

Antibiotics containing an aldehyde or a ketone group such as spiramycin I, tetracycline and erythromycin, did not show any $(M+2)^+$ or $(M+3)^+$ peaks under similar SI-MS conditions that we have examined. The hydrogenation reaction during SI-MS conditions, therefore, seems to be specific to quinone groups.

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