Collision-Induced Dissociation Actualized the H^+-Promoted Reaction as Observed *in Vitro*; Harman Formation from β-Carboline-Type Monoterpenoid Glucoindole Alkaloids

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The fragmentation from β-carboline-type monoterpenoid glucoindole alkaloids to harman, which is a hypothetical pathway to generate simple β-carbolines, was actualized in the collision-induced dissociation in MS.

Key words MS/MS; harman; β-carboline alkaloid; monoterpenoid glucoindole alkaloid; fragmentation; CID

Simple β-carboline alkaloids such as harman (**1**) are proved to be biogenetically formed, in general, from tryptophan and acetic acid or pyruvate.[1,2] Harmans co-occur sometimes in Rubiaceous plants accompanied with β-carboline-type monoterpenoid glucoindole alkaloids.

Previously, we observed that harman (**1**) could be obtained from lyaloside (**2**) or lyalosidic acid (**3**) by treatment with β-glucosidase in acetate buffer (pH 4.7). This finding led us to consider the fragmentation of a protonated compound as shown in Chart 1.[3] During our recent study[4,5] on Peruvian Uña de Gato (original plant: *Uncaria tomentosa*, Rubiaceae), we clarified the co-existence of a simple β-carboline alkaloid, harman (**1**), and β-carboline-type monoterpenoid alkaloids, *i.e.*, lyaloside (**2**) and 3,4-dehydro-5(*S*)-5-carboxystrictosidine (**4**). The above two findings supported the possibility of secondary formation of simple β-carboline alkaloids from β-carboline-type monoterpenoid glucoindole alkaloids through the fragmentation shown in Chart 1. We postulated that similar fragmentation would be actualized in the collision-induced dissociation (CID) in MS of these compounds when MH^+ was selected as the precursor ion.[6,7] In this paper, we describe the experiments of FAB-MS/MS (tandem mass spectrometry)[8] which resulted in the anticipated fragmentation of β-carboline-type monoterpenoid glucoindole alkaloids into simple β-carbolines.

We examined CID[9] of β-carboline-type monoterpenoid glucoindole alkaloids (**2**—**6**). The CID of lyaloside (**2**), a β-carboline-type alkaloid, showed two prominent product ions at *m/z* 365 $[MH-C_6H_{10}O_5]^+$, *m/z* 182 $[MH-C_{15}H_{21}O_9]^+$ and a weak ion at *m/z* 393 $[MH-C_5H_{10}O_4]^+$ when MH^+ (*m/z* 527) was selected as the precursor ion (Fig. 2). Lyalosidic acid (**3**), a carboxylic acid derivative of lyaloside (**2**), gave the same product ion at *m/z* 182 accompanied with the ions at *m/z* 379 $[MH-C_5H_{10}O_4]^+$ and *m/z* 351 $[MH-C_6H_{10}O_5]^+$ which correspond to the product ions of lyaloside (**2**), respectively. As anticipated, the product ion at *m/z* 182 corresponding to harman (**1**) was observed. Harman (**1**) in the CID would be formed through a homolytic cleavage of the C14—C15 bond by fragmentation of the protonated intermediates shown in Chart 2.

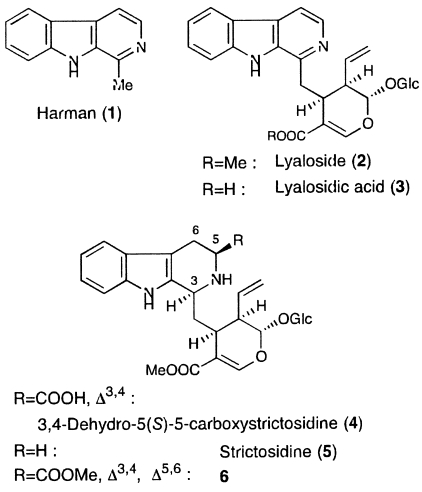

Fig. 1

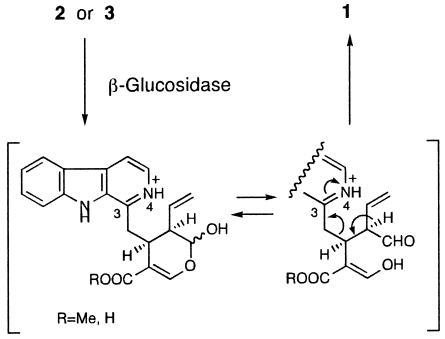

Chart 1

On the other hand, strictosidine (**5**), which is a tetrahydro-β-carboline-type compound, gave no ion corresponding to harman (**1**) in the CID. Furthermore, 3,4-dehydro-5(*S*)-5-carboxystrictosidine (**4**), a new alkaloid of Peruvian Uña de Gato, and 5-carbomethoxylyaloside (**6**),[10] which was synthesized from L-tryptophan methyl ester and secoxyloganin tetraacetate,[11] gave the product ions *m/z* 228 or *m/z* 240 corresponding to 5,6-dihydro-5(*S*)-5-carboxyharman or 5-carbomethoxyharman, respectively (Fig. 2). These data indicated that the 3,4-double bond in β-carboline-type alkaloids was essential for this type of fragmentation.

In conclusion, the fragmentation from β-carboline-type monoterpenoid glucoindole alkaloids to harman, which is a hypothetical pathway to generate the simple β-carbolines, was actualized in the CID measurement in MS. Further, we revealed that tandem mass spectrometry could be employed as the reaction-site of the H^+-promoted fragmentation reaction. By this method, it will be possible to predict the products and mechanism of various related reactions.

References and Notes

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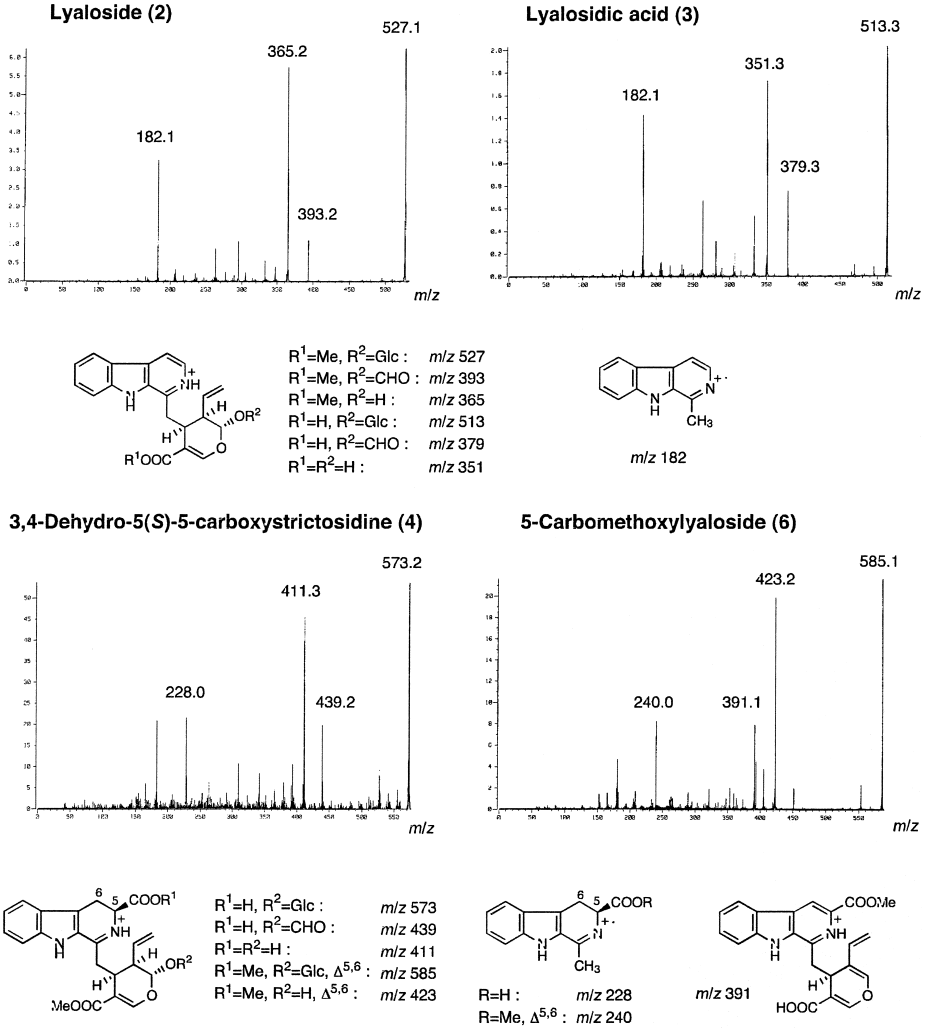

Fig. 2

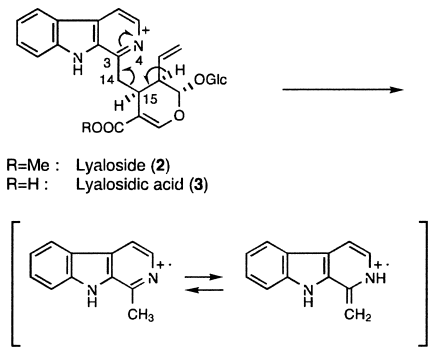

Chart 2

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9) CID experiments were performed with a four-sector (BE/BE) tandem mass spectrometer (JEOL JMS-700T) equipped with FAB source. Typical measurement conditions are as follows: acceleration voltage; 10.0 kV, matrix; NBA, collision gas; Xe, collision cell voltage; 0 V. Observed product ions. Lyaloside (**2**) *m/z*: 527 $[MH]^+$, 393, 365, 295, 182. Lyalosidic acid (**3**) *m/z*: 513 $[MH]^+$, 379, 351, 333, 263, 182. Strictosidine (**5**) *m/z*: 531 $[MH]^+$, 515, 171. 3,4-Dehydro-5(*S*)-5-carboxystrictosidine (**4**) *m/z*: 573 $[MH]^+$, 527, 439, 411, 228, 183. 5-Carbomethoxylyaloside (**6**) *m/z*: 585 $[MH]^+$, 553, 423, 391, 240, 180.
10) 5-Carbomethoxylyaloside (**6**) was prepared from L-tryptophan methyl ester and secoxyloganin tetraacetate. (i. secoxyloganin tetraacetate, EDCl, HOBT, dry CH_2Cl_2, then L-tryptophan methyl ester, ii. $POCl_3$, dry benzene, iii. 1 N NaOMe in MeOH, dry MeOH). Selected data for **6**. UV λ_{max} (MeOH): 350, 288, 250 (sh), 235 nm. ^{1}H-NMR (500 MHz, CD_3OD) δ: 8.75 (1H, s, H-6), 8.21 (1H, dd, J=8.2, 1.2 Hz, H-9), 7.61 (1H, dd, J=8.2, 1.2 Hz, H-12), 7.58 (1H, ddd, J=8.2, 8.2, 1.2 Hz, H-11), 7.52 (1H, d, J=1.0 Hz, H-17), 7.31 (1H, ddd, J=8.2, 8.2, 1.2 Hz, H-10), 5.90 (1H, ddd, J=17.1, 10.4, 8.8 Hz, H-19), 5.78 (1H, d, J=7.0 Hz, H-21), 5.02 (1H, dd, J=10.4, 1.5 Hz, H-18), 4.97 (1H, br d, J=17.1 Hz, H-18), 4.76 (1H, d, J=8.8 Hz, H-1′), 4.01 (3H, s, 5-COOMe), 3.30 (3H, s, 16-COOMe). ^{13}C-NMR (125 MHz, CD_3OD) δ: 169.5 (16-COOMe), 168.1 (5-COOMe), 154.2 (C-17), 146.0 (C-3), 142.6 (C-13), 138.2 (C-5), 137.3 (C-2), 135.6 (C-19), 129.8 (C-8), 129.4 (C-11), 123.0 (C-9), 122.6 (C-7), 121.6 (C-10), 119.3 (C-18), 117.3 (C-6), 113.3 (C-12), 111.0 (C-16), 100.2 (C-1'), 97.4 (C-21), 52.8 (5-COOMe), 51.6 (16-COOMe).
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