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HYDROLYTIC DEGRADATION OF β-CARBOLINE-TYPE MONOTERPENOID GLUCOINDOLE ALKALOIDS: A POSSIBLE MECHANISM FOR HARMAN FORMATION IN *Ophiorrhiza* AND RELATED RUBIACEOUS PLANTS

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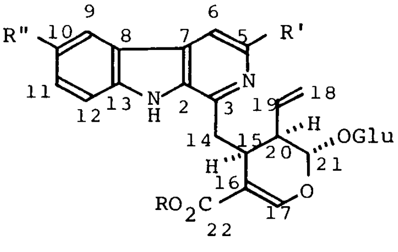
A new mechanism is proposed for harman formation which operates, probably secondarily, in *Ophiorrhiza* and other related Rubiaceous plants. It is based on the observed easy fragmentation of some β-carboline-type monoterpenoid glucoindole alkaloids by enzymatic cleavage of the glucoside bonds. Isolation of two new glucosidic alkaloids, lyalosidic acid and 10-hydroxylyalosidic acid, is also described.

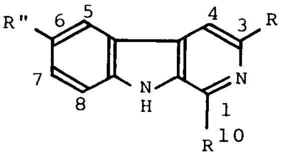
KEYWORDS—— *Ophiorrhiza japonica*; Rubiaceae; glucoindole alkaloid; new alkaloid; β-carboline; harman; 6-hydroxyharman; lyalosidic acid; 10-hydroxylyalosidic acid; biosynthesis

We recently reported the isolation and structure determination of the new type glucoindole alkaloids, ophiorines A and B, from *Ophiorrhiza* spp. (Rubiaceae).[1] Now two new glucosidic alkaloids, lyalosidic acid (**1**) and 10-hydroxylyalosidic acid (**2**), together with a known β-carboline alkaloid, 6-hydroxyharman (**3**), were isolated from *O. japonica* as described below.

Lyalosidic acid (**1**)[2] was obtained as an amorphous powder, $[\alpha]_D$ -151.7° (MeOH). On diazomethane methylation of (**1**), lyaloside (**4**)[3] was obtained. Another new glucoside, 10-hydroxylyalosidic acid (5-decarboxycordifolinic acid) (**2**),[4] was isolated as pale yellow prisms, mp > 300°C, $[\alpha]_D$ -127.7° (MeOH), λ_{max}^{MeOH}nm: 232, 248 (sh), 258(sh), 290(sh), 298, 310, 373, and 405(sh). Methylation of **2** with diazomethane gave 10-methoxylyaloside (**5**), mp 161-164°C. 6-Hydroxyharman (**3**),[5] pale yellow prisms, mp 276-278°C, was obtained as a non-glucosidic constituent. This alkaloid is the second simple β-carboline found in *O. japonica*, since Fujita and



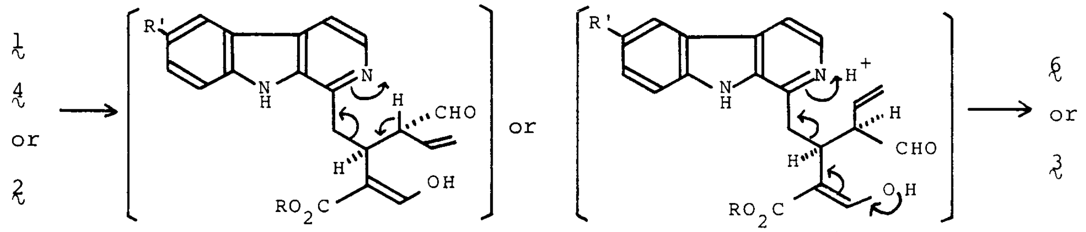
1: R= R'= R"= H
2: R= H R'= H R"= OH
4: R= Me R'=R"= H
5: R= Me R'= H R"= OMe
9: R=H R'=CO_2H R"=H



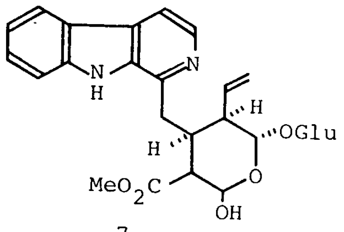
3: R= Me R'= H R"= OH
6: R= Me R'=R"= H
8: R= Me R'=CO_2Me R"= H

Sumi[6)] already isolated harman (**6**) from the same plant.

At this stage of work we attempted enzymatic hydrolysis of the glucosidic linkage of these alkaloids intending to obtain aglucones which would serve as the starting materials for biomimetic chemical conversions. This objective was not fruitful.[7)] Instead, a new type of fragmentation took place. Thus, lyaloside (**4**) (100 mg) was treated with emulsin (preparation from almond, Sigma Co.) in an acetate buffer (pH 4.7) at 37°C. After 5 days, the resulting wine-red reaction solution was neutralized with aqueous sodium carbonate and the product was extracted with chloroform. Harman (**6**) (8 mg) was obtained after purification of the product with Sephadex LH-20 and SiO_2 columns. Similarly **6** was obtained from **1**. When **2** was treated with the same enzyme, **3** was produced with 21% yield. These results, together with the above finding that the simple β-carbolines **3** and **6** coexist with the corresponding glucoalkaloids **2** and **1** in the same plant, strongly suggest a close relationship between these two types of constituents.



Wide distribution of harman (**6**) accompanying monoterpenoid indole alkaloids has been noted in Rubiaceous plants.[8)] In some plants, like *O. japonica*, coexistence of simple β-carbolines with the corresponding glucoindole alkaloids has been demonstrated, e.g., harman (**6**) and palinine (**7**) in *Palicourea alpa*,[9)] and 3-methoxycarbonylharman (**8**)[10)] and desoxycordifolinic acid (**9**)[11)] in *Nauclea diderrichii*. Therefore, in *Ophiorrhiza* and some other, if not all, Rubiaceous plants, formation of simple β-carbolines is considered to be made secondarily through β-carboline type monoterpenoid glucoindole alkaloids or their equivalents. This mechanism presents a striking contrast to the normal biosynthetic pathway of this class of alkaloids, in which a two-carbon unit (C_1-C_{10}) originates from acetate or pyruvate and the remaining part comes from tryptophan through tryptamine, as proven in Zygophyllaceae, Elaeagnaceae, Passifloraceae and other plant families.[12)]



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1) N. Aimi, T. Tsuyuki, H. Murakami, S. Sakai, and J. Haginiwa, Tetrahedron Lett., 26, 5299 (1985).

2) ^{13}C-NMR data for (**1**) (67.8 MHz, CD$_3$OD) δ : 36.0(C-15), 36.4(C-14), 46.2(C-20), 63.0(C-6'), 71.7(C-4'), 74.7(C-2'), 78.0(C-5'), 78.4(C-3'), 97.1(C-21), 100.4 (C-1'), 113.4(C-12), 114.9(C-6, C-16), 118.8(C-18), 121.2(C-10), 122.0(C-7), 122.9(C-9), 130.4(C-11), 131.1(C-8), 134.6(C-19), 135.8(C-13), 136.1(C-5), 143.1(C-2), 151.7(C-17), and 174.9(C-22).

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Lyaloside (**4**) in the present work has been identified with an authentic specimen which was kindly provided for us by Prof. André Cavé, Universite Paris Sud, in 1981.

4) ^{13}C-NMR data for (**2**) (67.8 MHz, CD$_3$OD) δ : 35.1(C-14), 35.6(C-15), 45.9(C-20), 62.9(C-6'), 74.7(C-2'), 78.0(C-5'), 78.5(C-3'), 97.1(C-21), 100.3(C-1'), 106.6 (C-9), 113.6(C-16), 114.1(C-12), 115.3(C-6), 119.3(C-18), 121.6(C-11), 122.6 (C-7), 131.6(C-8), 132.3(C-5), 135.7(C-19), 136.3(C-13), 138.3(C-2), 143.8(C-3), 152.3(C-17), 153.0(C-10), and 173.0(C-22).

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