HUNTERIOSIDE, FIRST BIOSE BOUND MONOTERPENOID INDOLE ALKALOID FROM *HUNTERIA ZEYLANICA*

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A novel glycosidic alkaloid, hunterioside, together with strictosidinic acid was isolated from *Hunteria zeylanica*, and the structure was found to be 6′-α-D-glucoside of strictosidinic acid. This isolation is the first finding of a bioside congener of monoterpenoid indole alkaloid glucosides.

KEYWORDS hunterioside; strictosidinic acid; glycosidic alkaloid; secoiridoid; *Hunteria zeylanica*; Apocynaceae

Well over 1,400 different monoterpenoid indole alkaloids are distributed in nature.[1] They have attracted the attention of phytochemists and synthetic chemists all over the world for their fascinating diversity of molecular structures and for their remarkable pharmacological activity. Clarification of biosynthetic pathways of this group of alkaloids has been pursued for many years, and particular attention has been focused on the early stage. Until now a glucosidic alkaloid, strictosidine (**1**), has been rigorously proved to be the true and universal intermediate to all members of this group. Recently Zenk and Kutchan[2] succeeded in purification and cloning of strictosidine synthase, an enzyme which catalyzes formation of strictosidine (**1**) from tryptamine (**2**) and secologanin (**3**). This work was noted as the first attainment of cDNA cloning of an alkaloid-forming enzyme.


(**2**)

(**3**) R = Me
(**4**) R = H

(**1**) R = Me
(**5**) R = H
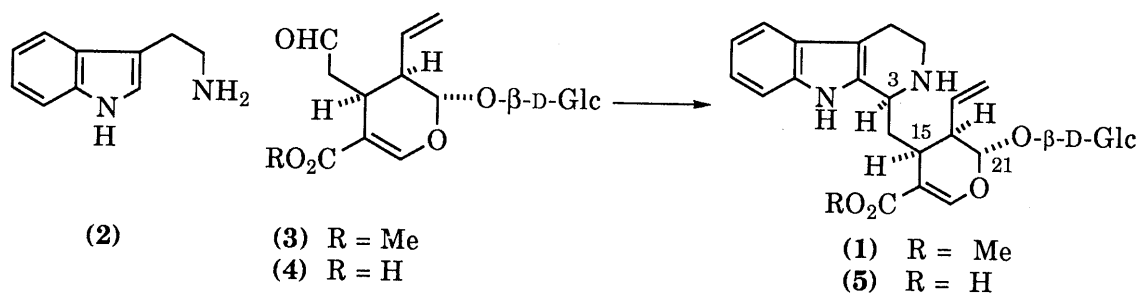
Glycosidic alkaloids as exemplified by strictosidine (**1**) form an independent structural group in the whole monoterpenoid indole alkaloids, and to this date over 20 alkaloids are known.[3] All members hitherto known carry one unit of D-glucose at hemiketal oxygen on C-21, and thus the non-tryptamine parts are believed to be of secologanin origin, as was the case with strictosidine (**1**). In this paper we describe isolation of a novel monoterpenoid indole alkaloid glycoside, hunterioside (**6**), from *Hunteria zeylanica* (Apocynaceae). We proved that **6** has one additional molecule of glucose at the C-6′ hydroxyl group of strictosidinic acid (**5**). This is the first finding of biose-carrying monoterpenoid indole alkaloid, the non-tryptamine part of which probably originates from corresponding biosidic secoiridoid.

An EtOH extract of the stem bark of *Hunteria zeylanica*, collected in south Thailand, was shaken with dilute hydrochloric acid, and the obtained aqueous layer was basified with ammonia. After being washed with CHCl$_3$ the aqueous layer was extracted with n-BuOH to give a syrupy residue. Flash column chromatography (SiO$_2$) and preparative TLC purification enabled us to obtain two glycosidic components, BHZ-1 and BHZ-2.

BHZ-1 was shown to be strictosidinic acid (**5**)[4,5] through comparison of the ^{13}C-NMR spectrum with that of a sample prepared by *in vitro* condensation of tryptamine with secologanic acid (**4**).

BHZ-2, now named hunterioside (6),[6] has the molecular formula of $C_{32}H_{42}N_2O_{14}$ as was revealed by a high-resolution FAB-MS measurement . This observation indicates that the molecule of hunterioside is bigger than strictosidinic acid (5) by an increment of $C_6H_{10}O_5$, a molecular unit corresponding to one additional hexose. It was quite obvious that the aglycone part of 6 was same as that of strictosidinic acid (5) from the finding that the ^{13}C-NMR spectra of both compounds showed almost superimposable peaks ascribable to the aglycones. The presence of two sugars in the molecule of 6 was revealed by two anomeric proton NMR signals at δ 4.91 (d, J=3.7 Hz) and δ 4.87 (d, J=8.1 Hz), and by the corresponding carbon signals at δ 99.6 and δ 100.3 as well. To identify the composing sugars we hydrolyzed 6 with dilute hydrochloric acid. Sodium borohydride reduction of the resulting sugar gave the corresponding alditol, which was then acetylated. The obtained alditol acetate (7) [7] was a homogeneous single compound and was identified as sorbitol hexaacetate, the reference sample of which was prepared from D-glucose. The observed optical rotation $[\alpha]_D$ +9.4° of our degradation product (7) indicated that the absolute configuration of the alditol thus obtained is D.[7] This result accordingly proved that both of the two sugars in 6 are D-glucose.
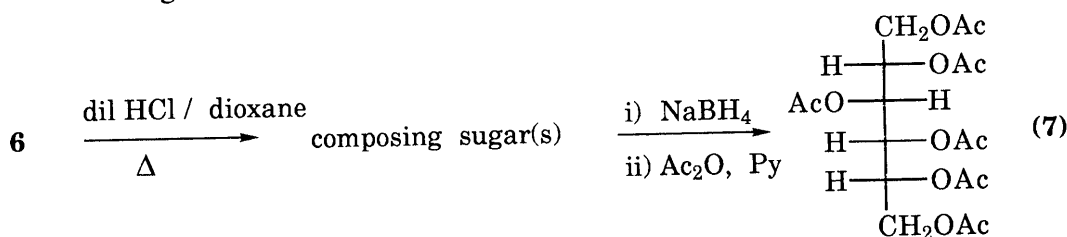

For the following reasons we concluded that the two glucose units form a bioside linkage, α-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (isomaltoside), and this moiety is connected to C-21 hemiketal oxygen on the aglycone part.
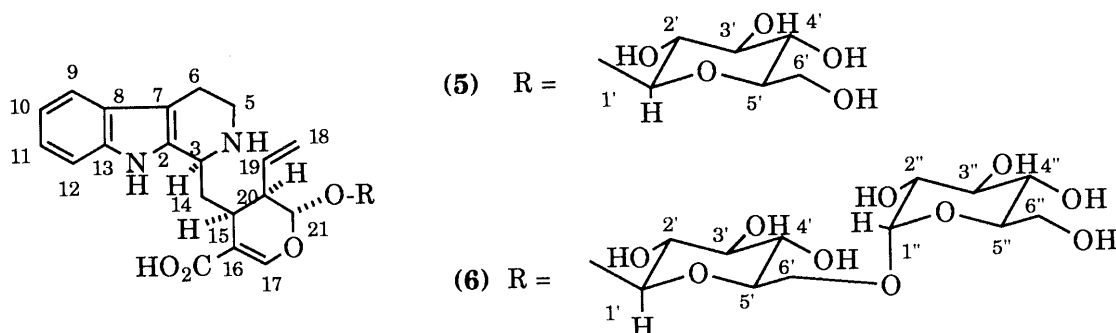
Supporting evidence for the depicted structure includes, among others, the following observations: (i) a HMBC measurement showed the presence of long range C-H coupling between C-21 (δ 97.0) and H-1' (δ 4.87) and between H-21 (δ 5.70) and C-1' (δ 100.3); (ii) the doublet signal due to H-1' showed a trans diaxial coupling constant of J=8.1 Hz, proving β-orientation of the glycosidic bond; (iii) ^{13}C-chemical shifts of C-6' (δ 67.2) and C-5' (δ 76.5) show reasonable values as effected by an introduced substituent at C-6' hydroxyl group;[8] (iv) long range C-H couplings were observed by HMBC between C-6' and H-1" (δ 4.91), and between H-6' (δ 3.83, 3.95) and C-1" (δ 99.6); (v) H-1" has a coupling constant of J=3.3 Hz, indicating an α-axial glucosidic linkage at C-1" position; and (vi) chemical shifts of the carbons C-1" - C-6" are reasonable for an α-substituted glucopyranose.

Hunterioside (6) was thus deduced to be strictosidinic acid 6'-α-D-glucoside. This was the first finding of a natural biose congener of monoterpenoid indole alkaloid glycosides. We are interested in knowing whether 6, or its methyl ester, has any possibility of playing an active role in indole alkaloid biosynthesis as strictosidine (1) does. We are also interested in looking for secoiridoids 8 or 9 in nature. These are bioside analogues of secologanin (3) and secologaninic acid (4) and regarded to be presumed biosynthetic
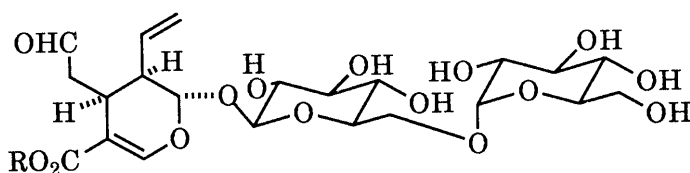
(8) R = H

(9) R = CH₃

precursors of **6**. To our knowledge neither of them has been reported yet.

REFERENCES AND NOTES

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2) T. M. Kutchan, *Phytochem.*, **32**, 493 (1993), and references cited therein.

3) R. S. Kapil and R. T. Brown, "The Alkaloids", Vol **17**, ed. by R. H. F. Manske, R. G. A. Rodrigo, Academic Press Inc., New York, 1979, pp. 545-588.

4) BHZ-1 (Strictosidinic acid) (**5**) ; ^{13}C-NMR (125MHz, CD3OD) , δ: 19.5 (C-6), 34.0 (C-15), 35.1 (C-14), 42.9 (C-5), 45.6 (C-20), 52.2 (C-3), 63.1 (C-6'), 71.8 (C-4'), 74.7 (C-2'), 78.0 (C-3'), 78.7 (C-5'), 96.6 (C-21), 100.3 (C-1'), 107.3 (C-7), 112.3 (C-12), 113.6 (C-16), 118.9 (C-9), 119.0 (C-18), 120.5 (C-10), 123.3 (C-11), 127.5 (C-8), 130.5 (C-2), 136.2 (C-19), 138.1 (C-13), 153.3 (C-17), 176.0 (CO).

5) K. T. D. De Silva, D. King, G. N. Smith, *Chem. Commun.*, **1971**, 908.
Detail of spectroscopical studies on this and related compounds in our laboratory will be reported elsewhere.

6) Hunterioside (BHZ-2) (**6**); Amorphous powder, $C_{32}H_{42}N_2O_{14}$. High resolution FAB-MS; Found : m/z 679.2709; Calcd. for $C_{32}H_{43}N_2O_{14}$: m/z 679.2715 (M + H)$^+$. ^{1}H-NMR (500 MHz, CD3OD) δ; 2.13 (ddd, *J*=14.4, 14.4, 4.0 Hz, H-14), 2.30 (ddd, *J*= 14.6, 14.6, 3.5 Hz, H-14), 2.67 (ddd, *J*= 9.0, 9.0, 6.0 Hz, H-20), 2.91 - 2.95 (2H, m, H-6, H-15), 3.01 - 3.08 (m, H-6), 3.19 (ddd, *J*= 12.5, 12.5, 4.9 Hz, H-5), 3.27 (dd, J= 9.3, 8.0 Hz, H-2'), 3.41 - 3.48 (4H, m, H-3', H-4', H-2", H-4"), 3.60 (m, H-5'), 3.70 - 3.81 (5H, m, H-5, H-3", H-5", H-6", H-6"), 3.83 (dd, *J*= 11.6, 2.1 Hz, H-6'), 3.95 (dd, *J*= 11.4, 5.0 Hz, H-6'), 4.40 (br.d, *J*= 11.7 Hz, H-3), 4.87 (d, *J*= 8.1 Hz, H-1'), 4.91 (d, *J*= 3.3 Hz, H-1"), 5.22 (d, *J*= 10.7 Hz, H-18), 5.29 (d, *J*= 17.3 Hz, H-18), 5.70 (d, *J*= 9.3 Hz, H-21), 5.83 (ddd, *J*= 17.8, 10.6, 7.7 Hz, H-19), 7.04 (dd, *J*= 7.5, 7.5, 1.0 Hz, H-10), 7.14 (ddd, *J*= 7.5, 7.5, 1.0 Hz, H-11), 7.44 (2H, d, *J*= 8.3 Hz, H-9, H-12), 7.52 (s, H-17).
^{13}C-NMR (125Hz, CD3OD), δ: 19.4 (C-6), 33.5 (C-15), 34.7 (C-14), 43.0 (C-5), 45.7 (C-20), 52.3 (C-3), 62.2 (C-6"), 67.2 (C-6'), 71.2 (C-4'), 71.3 (C-4"), 73.4 (C-2"), 73.4 (C-5"), 74.4 (C-2'), 74.8 (C-3"), 76.5 (C-5'), 77.7 (C-3'), 97.0 (C-21), 99.6 (C-1"), 100.3 (C-1'), 107.4 (C-7), 112.6 (C-12), 113.9 (C-16), 119.0 (C-9), 119.6 (C-18), 120.5 (C-10), 123.4 (C-11), 127.3 (C-8), 130.3 (C-2), 135.8 (C-19), 138.0 (C-13), 153.0 (C-17), 176.2 (CO).

7) **7** (D-Sorbitol hexaacetate); syrup. $C_{18}H_{26}O_{12}$. High resolution FAB-MS; Found: m/z 435.1404; Calcd. for $C_{18}H_{27}O_{12}$: m/z 435.1403 (M + H)$^+$. [α]$_D$ +9.4 ° (c= 0.325, acetone) (Lit. [α]$_D$ +9.5 ° (c= 1.0, acetone) (Beilstein, Handbuch der Organische Chemie: **2**, 150; II 163). ^{13}C-NMR (125 MHz, CDCl3), δ; 20.46, 20.61, 20.65, 20.66, 20.70, 20.72 (6 x CO-<u>C</u>H3); 61.42, 61.79, 68.36, 68.58, 68.70, 69.30 (C-1 - 6); 169.72, 169.74, 169.80, 169.90, 170.35, 170.46 (6 x <u>C</u>O-CH3).

8) O. Tanaka, *Yakugaku Zasshi*, **105**, 323 (1985).