HUNTERIOSIDE B, A DISACCHARIDE CARRYING MONOTERPENOID INDOLE ALKALOID, FROM *HUNTERIA ZEYLANICA*

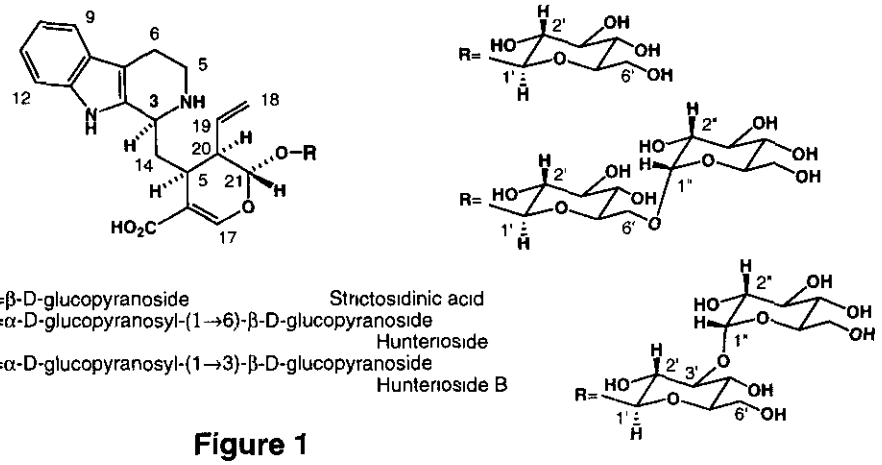
Hiromitsu Takayama[a]*, Sanan Subhadhirasakul[b], Osamu Ohmori[a], Mariko Kitajima[a], Dhavadee Ponglux[c], and Norio Aimi[a]*

[a]Faculty of Pharmaceutical Sciences, Chiba University, 1-33, Yayoi-cho, Inage-ku, Chiba 263, Japan, [b]Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songlka, 90112, Thailand, [c]Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok,10330, Thailand

Abstract - A new glycosidic indole alkaloid, hunterioside B (**3**), was isolated from *Hunteria zeylanica* collected in south Thailand, and the structure was elucidated to be a strictosidinic acid 3'-α-D-glucoside using spectroscopic analysis.

The chemical investigations on the components of the tropical Apocynaceous plant, *Hunteria zeylanica* growing in Thailand, have resulted in the isolation of several known and new monoterpenoid indole alkaloids.[1,2] In addition, the pharmacological studies of this plant have been carried out, which have revealed that the alkaloidal fraction obtained from the stem bark exhibited antinociceptive, antipyretic and anti-inflammatory activities.[3] These new chemical and pharmacological findings have stimulated us to pursue the alkaloidal constituents in the stem bark of *H. zeylanica*. In this paper, we describe the isolation and structure elucidation of a new glycoside monoterpenoid indole alkaloid.

Together with two known glycosidic alkaloids, strictosidinic acid (**1**) and hunterioside (**2**),[1] a new compound (**3**)[4] was isolated as a minor component from the highly polar alkaloid fraction [R_f values on SiO_2 thin layer chromatography: (**1**) 0.38, (**2**) 0.13, (**3**) 0.18, solvent system: $CHCl_3$:MeOH:H_2O = 6:3:0.5]. The presence of an indolic nucleus and also a conjugated enol ether chromophore in **3** was shown by the typical UV spectrum. The high resolution FABMS of **3** demonstrated the molecular composition of $C_{32}H_{42}N_2O_{14}$ by observation of the peaks at 701.2524 $(M+Na)^+$ and 679.2725 $(M+H)^+$. Furthermore, characteristic fragments at m/z 539 and 517, which correspond to the peaks that resulted



1 : R=β-D-glucopyranoside Strictosidinic acid
2 : R=α-D-glucopyranosyl-(1→6)-β-D-glucopyranoside Hunterioside
3 : R=α-D-glucopyranosyl-(1→3)-β-D-glucopyranoside Hunterioside B

Figure 1

from the respective elimination of a $C_6H_{10}O_5$ unit from the parent peaks above, were also observed. The ^{1}H-NMR spectrum of **3**, which exhibited characteristic signals due to four aromatic protons in its A ring, three olefin protons on the vinyl group, and three acetal protons at δ 5.78, 5.18, and 4.85 as well as signals due to other aliphatic protons, was very similar to that of hunterioside (**2**).[1] Furthermore, the ^{13}C-NMR spectrum showed the presence of twenty carbons corresponding to the aglycone part of strictosidinic acid (**1**) and of the two hexose units. These peaks are almost superimposable and ascribable to hunterioside (**2**). From these data, the structure of the new compound (**3**) could be deduced to be, just like hunterioside (**2**), a glucopyranoside of strictosidinic acid (**1**).

After complete assignment of all the protons and carbons using the HH-COSY, HSQC, HMBC and 1D-HOHAHA spectra, the connection mode and the stereochemistry of the two sugars in **3** were determined as follows. The observation of three-bond coupling between C21 (δ 96.7) and H1' (δ 4.85) and between H21 (δ 5.78) and C1' (δ 100.5) demonstrated that the anomeric carbon in the first glucopyranose was connected to the C21 hemiacetal oxygen on the aglycone part. The proton at C1' is coupled to H2' with a coupling constant of 8.1 Hz, proving a β-orientation for the first glycosidic bond. The chemical shift at C3' (δ 86.8) in the first glucopyranose was shifted downfield by 8.8 ppm compared with that of **1** (see Table), indicating that a substituent was introduced on the oxygen at the C3' position. The long-range coupling between C3' (δ 86.8) and H1" (δ 5.18) and between H3' (δ 3.55) and C1" (δ 101.6) demonstrated that the C3' oxygen was connected to the hemiacetal carbon (C1") in the second glucopyranose. The proton at C1" has a coupling constant of J=3.9 Hz with H2", indicating an α-axial glucosidic linkage at the C1" position. The chemical shifts of the six carbons in the second sugar are reasonable for an α-

hunterioside (**2**) and other glucosidic monoterpenoid indole alkaloids. Thus the two glucose units in **3** constitute a bioside linkage in the mode of α-D-glucopyranosyl-(1→3)-β-D-glucopyranoside.

As shown in the Figure 2, the CD spectra of **1**, **2**, and **3** displayed almost superimposable curves. Therefore, these three compounds have the same absolute configuration including the stereochemistry at the C3 position.

In conclusion, the new indole alkaloid, now named hunterioside B, is a strictosidinic acid 3'-α-D-glucoside, which is a second example of biose bouned monoterpenoid indole alkaloids. Synthetic studies on these novel biose bounded monoterpenoid indole alkaloids are currently in progress in our laboratory.

Table ^{13}C-NMR data of the compounds (**1**, **2** and **3**)

carbons	1	2	3	carbons	1	2	3
2	130.5	130.3	130.2	1'	100.3	100.3	100.5
3	52.2	52.3	52.4	2'	74.7	74.4	73.4
5	42.9	43.0	43.2	3'	*78.0*	*77.7*	*86.8*
6	19.5	19.4	20.0	4'	71.8	71.2	71.7
7	107.3	107.4	107.5	5'	78.7	76.5	78.3
8	127.5	127.3	127.7	6'	*63.1*	*67.2*	*63.0*
9	119.0	119.0	119.0	1"	-	99.6	101.6
10	120.5	120.5	120.4	2"	-	73.4	74.1
11	123.3	123.4	123.1	3"	-	74.8	75.2
12	112.3	112.6	112.2	4"	-	71.3	71.8
13	138.1	138.0	138 2	5"	-	73.4	73.9
14	35.1	34.7	35 5	6"	-	62.2	62.6
15	34.0	33.5	34.0				
16	113.6	113.9	114.3				
17	153.3	153.0	152.6				
18	118.9	119.6	118.8				
19	136.2	135.8	136.4				
20	45.6	45.7	46.0				
21	96.6	97.0	96.7				
CO	176.0	176.2	175.9				

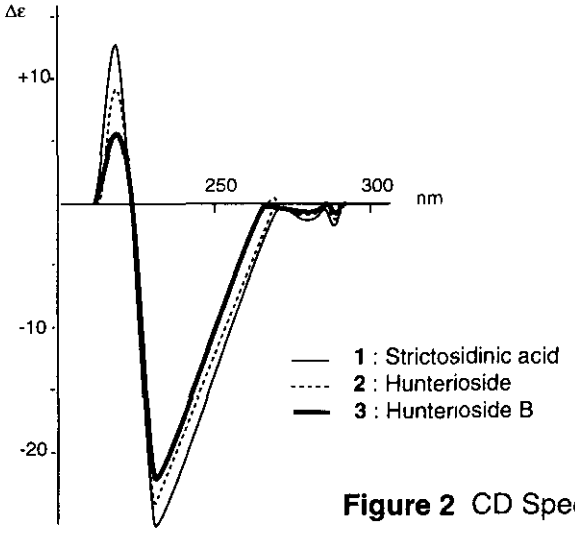


Figure 2 CD Spectra

ACKNOWLEDGMENTS

This work was supported in part by Grants-in-Aid (No. 08680627) for Scientific Research from the Ministry of Education, Science, Sports, and Culture, Japan.

REFERENCES AND NOTES

1. S. Subhadhirasakul, N. Aimi, H. Takayama, D. Ponglux, and S. Sakai, *Chem. Pharm. Bull.*, 1994, **42**, 991.

2. (a) H. Takayama, S. Subhadhirasakul, J. Mizuki, M. Kitajima, N. Aimi, D. Ponglux, and S. Sakai, *Chem. Pharm. Bull.*, 1994, **42**, 1957. (b) S. Subhadhirasakul, H. Takayama, Y. Miyabe, N. Aimi, D. Ponglux, and S. Sakai, *Chem. Pharm. Bull.*, 1994, **42**, 2645. (c) S. Subhadhirasakul, H. Takayama, Y. Miyabe, M. Kitajima, D. Ponglux, S. Sakai, and N. Aimi, *Heterocycles*, 1995, **41**, 2049.

3. (a) W. Reanmongkol, K. Matsumoto, H. Watanabe, S. Subhadhirasakul, and S. Sakai, *Biol. Pharm. Bull.*, 1994, **17**, 1345. (b) W. Reanmongkol, K. Matsumoto, H. Watanabe, S. Subhadhirasakul, H. Takayama, and S. Sakai, *Biol. Pharm. Bull.*, 1995, **18**, 33. (c) W. Reanmongkol, M. Tohda, K. Matsumoto, S. Subhadhirasakul, H. Takayama, S. Sakai, and H. Watanabe, *Biol. Pharm. Bull.*, 1995, **18**, 910. (d) P. Leewannich, M. Tohda, K. Matsumoto, S. Subhadhirasakul, H. Takayama, N. Aimi, and H. Watanabe, *Biol. Pharm. Bull.*, 1996, **19**, 394.

4. Hunterioside B (**3**); colorless amorphous solid, UV λmax nm (EtOH); 221, 272, 289. CD $\Delta\varepsilon^{23}$ (nm) (c=0.18 x 10^{-3} *mol/L*, MeOH); 0 (293), -1.29 (289), -0.41 (285), -0.95 (281), -0.41 (265), -22.23 (231), 0 (222), +5.13 (217), 0 (211). High Resolution FABMS; Found m/z 701.2524, Calcd for $C_{32}H_{42}N_2O_{14}Na$ $(M+Na)^+$, m/z 701.2533, Found m/z 679.2725. Calcd for $C_{32}H_{43}N_2O_{14}$ $(M+H)^+$, m/z 679 2715. FAB-MS (NBA); 701 $(M+Na)^+$, 679 $(M+H)^+$, 539, 517. ^{1}H-NMR (500 MHz, CD_3OD) δ; 7.52 (1H, s, H-17), 7.43 (1H, d, J= 7.8 Hz, H-9), 7.30 (1H, dd, J=7.8, 1.0 Hz, H-12), 7 11 (1H, td, J=7.8, 1.0 Hz, H-11), 7.02 (1H, td, J=7.8, 1.0 Hz, H-10), 5.85 (1H, ddd, J=17.5, 11.6, 7.5 Hz, H-19), 5.78 (1H, d, J=9.3 Hz, H-21), 5 31 (1H, br d, J=17.5 Hz, H-18), 5.19 (1H, br d, J=11.6 Hz, H-18), 4.40 (1H, br d, J=11.7 Hz, H-3), 3.70-3.64 (4H, m, H-5, H-6', H-6", H-3"), 3.20 (1H, td, J=12.2, 5.1 Hz, H-5), 3.03-2.93 (3H, m, H-6, H-6, H-15), 2.68 (1H, m, H-20), 2.32 (1H, td, J=14.6, 2.4 Hz, H-14), 2.09 (1H, td, J=14.6, 4.8 Hz, H-14), 4.85 (1H, d, J=8.1 Hz, H-1'), 3.99 (1H, dd, J=11.7, 1.9 Hz, H-6'), 3 55 (1H, t, J=8.8 Hz, H-3'), 3.51 (1H, t, J=9.3 Hz, H-4'), 3.42 (1H, m, H-5'), 3.34 (1H, t, J=8.1 Hz, H-2'), 5.18 (1H, d, J=3.9 Hz, H-1"), 3.94 (1H, ddd, J=10.0, 5.4, 2.4 Hz, H-5"), 3.82 (1H, dd, J=11.7, 2.4 Hz, H-6"), 3.44 (1H, dd, J=9.5, 3.9 Hz, H-2"), 3.30-3.26 (1H, concealed by solvent peaks, H-4"). ^{13}C-NMR (125 MHz, CD_3OD) δ; see the Table.

Received, 22nd April, 1997