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STRUCTURE OF OPHIORINES A AND B; NOVEL TYPE GLUCO INDOLE ALKALOIDS ISOLATED FROM OPHIORRHIZA SPP.

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Abstract: Two novel type gluco indole alkaloids, ophiorines A and B, were found in Rubiaceous plants, *Ophiorrhiza japonica* Bl. and *O. kuroiwai* Mak., and their structures were elucidated by chemical and spectroscopical methods.

In 1967, Fujita et al.[1] reported the isolation of harman from *Ophiorrhiza japonica* Bl. We were interested in a closer investigation of the constituents of this plant, since recently pharmacologically active alkaloids were isolated from the same genus.[2]

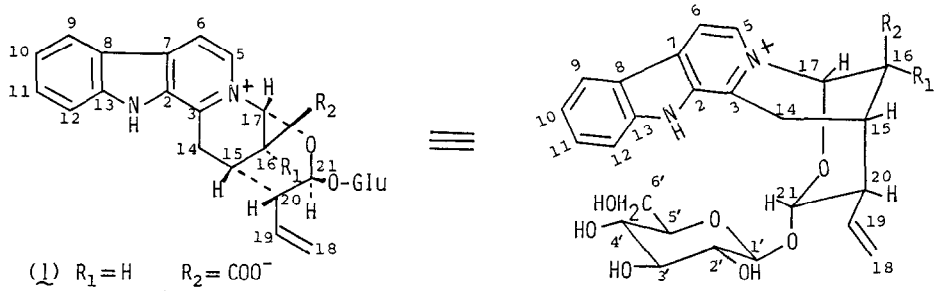
The extract of the leaves of *O. japonica* was partitioned between chloroform and water. From the water layer two novel betaine type indole alkaloids, ophiorines A (1) and B (2), were isolated. The same alkaloids, accompanied with several other constituents,[3] were also found in *O. kuroiwai* Mak. which grows in Ishigaki and other south-west islands of Okinawa Prefecture.

Ophiorine A (1) was isolated as pale yellow needles, mp 217-219°C, $[\alpha]_D$ +51.0°. The UV spectrum having the absorption maxima at 254, 310 and 365 nm indicated the β-carbolinium chromophore. Addition of alkali caused the expected shifts to the absorption of anhydrobase (λ_{max} 284, 324, 331 and 425 nm).[4] The FAB-MS data (m/z 513, M^++1) and the elemental analysis proved its molecular formula to be $C_{26}H_{28}O_9N_2$.

The ^{1}H-NMR spectrum (D_2O) of (1) showed two protons of C-ring of β-carboline at δ 8.47 (H-5) and δ 8.26 (H-6) along with four aromatic protons on the A ring at δ 7.98 (H-9), δ 7.28 (H-10), δ 7.62 (H-11) and δ 7.45 (H-12). Three protons on a vinyl side chain were observed at δ 5.45 (H-18a), δ 5.46 (H-18b) and δ 5.98 (H-19). The anomeric proton, H-1', was observed at δ 4.55 as a

doublet with a coupling constant of 7.9 Hz, indicating β-configuration of the glycosidic linkage. The acetal structure of the aglycone side was proved by another acetal proton at δ 4.72 as a doublet. The coupling constant, J=9.9 Hz, demonstrated trans diaxial arrangement between H-21 and H-20. A characteristically highly deshielded signal was observed at δ 6.67 as a broad singlet. This signal was assignable to H-17, the proton on a carbon holding both a positively charged nitrogen and an ethereal oxygen. (Table 1).

The ^{13}C-NMR of (1) was measured in D_2O. (Table 2). The composing sugar was clearly demonstrated to be glucose. Three carbons, each carrying two hetero atoms, were observed at δ 101.8 (C-1'), δ 98.2 (C-21) and δ 91.7 (C-17). All these and other data indicated that the structure of ophiorine A (1) was shown by the formula 1, stereochemistry of which was clarified as follows.



(1) R_1=H R_2=COO^-
(2) R_1=COO^- R_2=H

Chart 1

When (1) was methylated with diazomethane in methanol, simultaneous retro-Michael reaction occurred to give a known gluco indole alkaloid, lyaloside(3).[5] This observation allowed us to deduce stereochemical configurations at C_{15} (and therefore, C_{17}), C_{20} and C_{21} as depicted in the above structure (1). Stereochemistry of the last chiral centre, C_{16}, is described below in connection with the structure of the second alkaloid, ophiorine B (2).

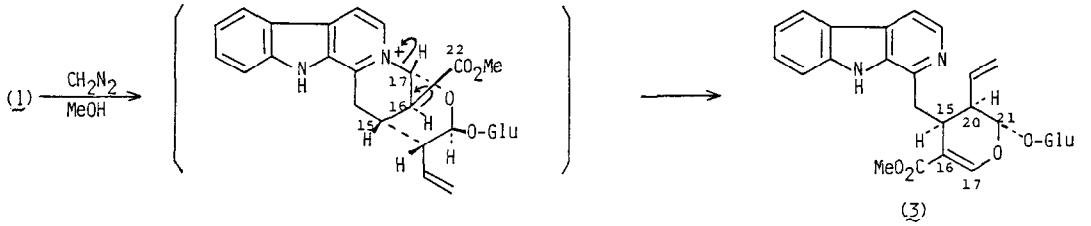


Chart 2

Ophiorine B (2) was isolated as pale yellow needles, mp 188-191°C, $[\alpha]_D$ +18.2°. The ^{1}H- and ^{13}C-NMR data are shown in Table 1 and 2, respectively. We found that (2) was a stereoisomer of (1) concerning C_{16}, since methylation of (2) with diazomethane and subsequent work-up also gave rise to lyaloside (3). Assignment of configuration at C_{16} was made on the basis of ^{13}C-NMR evidence.

^{1}H-NMR(270MHz, D_2O)

	Ophiorine A (1)		Ophiorine B (2)	
	δ(ppm)	J(Hz)	δ(ppm)	J(Hz)
H-5	8.47 (d)	6.3	8.49 (d)	6.6
H-6	8.26 (d)	6.3	8.41 (d)	6.6
H-9	7.98 (d)	7.9	8.18 (d)	7.9
H-10	7.28 (br-t)	7.9	7.40 (br-t)	ca.8.0
H-11	7.62 (br-t)	ca.8.0	7.76 (br-t)	ca.8.0
H-12	7.45 (d)	8.5	7.66 (d)	8.6
H-14a	3.68(1H,br-s)		3.69 (dd)	19.0,7.2
H-14b			3.85 (d)	19.0
H-15	3.27 (m)		3.22 (m)	
H-16	3.62 (t-like)		3.19 (t-like)	
H-17	6.67 (br-s)		6.69 (br-s)	
H-18a	5.45 (d)	17.8	5.39 (br-d)	17.8
H-18b	5.46 (d)	10.6	5.40 (br-d)	10.5
H-19	5.98 (ddd)	17.8,10.6 6.3	5.91 (ddd)	17.8,10.5 5.9
H-20	2.98 (m)		2.84 (m)	
H-21	4.72 (d)	9.9	4.68 (d)	9.6
H-1'	4.55 (d)	7.9	4.48 (d)	7.9
H-2'	3.28 (t-like)		3.19 (t-like)	
H-3'	3.44 (t-like)		3.40 (t-like)	
H-4'	3.28 (t-like)		3.20 (t-like)	
H-5'	3.16 (ddd)	8.9,5.3 2.3	3.16 (ddd)	10.4,5.9 ca.2.0
H-6'a	3.42 (dd)	12.5,5.3	3.39 (dd)	10.5,5.9
H-6'b	3.54 (dd)	12.5,2.3	3.58 (dd)	10.5,ca.2.0

Table 1

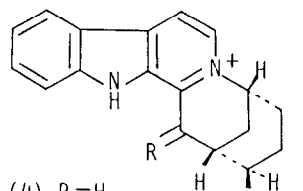
^{13}C-NMR(67.8MHz, D_2O)

	(1)	(2)
	δ(ppm)	δ(ppm)
C-2	134.8	135.6
C-3	146.4	146.4
C-5	136.9	136.8
C-6	135.9	135.8
C-7	140.2	140.4
C-8	122.1	121.9
C-9	124.6	124.6
C-10	119.2	119.2
C-11	125.5	125.5
C-12	115.4	115.3
C-13	135.8	134.7
C-14	26.0	29.1
C-15	33.0	33.0
C-16	49.8	47.4
C-17	91.7	91.5
C-18	121.9	122.3
C-19	134.8	134.8
C-20	48.8	46.0
C-21	98.2	98.2
C-22	176.9	177.4
C-1'	101.8	102.0
C-2'	75.4[a]	75.4[b]
C-3'	78.8[a]	78.9[b]
C-4'	72.2[a]	72.2[b]
C-5'	78.4[a]	78.3[b]
C-6'	63.3	63.2

a,b; Assignments are interchangeable in the same column.

Table 2

The signal due to C-20 of (1) was observed at δ 48.8, while that of (2) was at δ 46.0. This upfield shift of 2.8 ppm in (2) was ascribable to γ-effect due to C_{16}-α-substituent which is situated at a 1,3-diaxial position to the C_{20} hydrogen. Therefore, assignment of configuration at C_{16} of (1) and (2) was made as shown in the formula in Chart 1. Another proof for this stereochemical assignment was given by ^{1}H-NMR evidence. While all other peaks are very similar each other, a characteristic difference between (1) and (2) was observed in the peak positions of H-16. (Table 1). The down field shift of H-16 in (1) was reasonably explained in terms of the deshilding anisotropic effect caused by the aromatic ring, in the plane of which this proton lies.



(4) R=H_2
(5) R=O

It is interesting to note that C-14 signal of (1) disappeared almost completely when ^{13}C-NMR was taken in D_2O at an elevated temperature (60°C) or

after keeping the solution for several days at room temperature. This was obviously caused by easy H/D exchange through the scheme shown in Chart 3. In fact, in these cases H-14 signals in the ^{1}H-NMR spectra also disappeared. A similar observation has been reported by Hesse et al. for melinonine E (4), a new type alkaloid isolated from a Strychnos sp.[6]

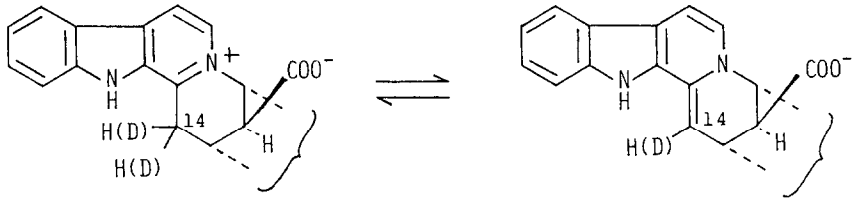


Chart 3

More than 20 gluco indole alkaloids have been found in nature.[7] Among them ophiorines A (1) and B (2) are unique for possessing $N_{(b)} - C_{17}$ linkage and β-carbolinium type structure. Biogenetic relationship between these alkaloids and melinonine E (4) or closely related strychnoxanthine (5)[8] arouses our interest.

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