Absolute Stereochemistry of (−)-Preorixine and Related Compounds

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The absolute stereochemistry of the C-2′ position of (−)-preorixine, postulated to be an important biosynthetic precursor of various quinoline alkaloids, was assigned as *R* by the combination of its chemical transformation and the extended Mosher method. The stereochemistry of the C-2′ position of (+)-orixine was also concluded to be *R*, establishing that no inversion occurred when (−)-preorixine was transformed into (+)-orixine.

Key words *Orixa japonica*; Rutaceae; (−)-preorixine; (+)-orixine; 3′-*O*-methylorixine; extended Mosher method

Orixa japonica (Rutaceae) is a shrub widely distributed in Japan, Korea and China. The stems and leaves of this plant were formerly used in Japan as an insecticide for livestock, and from the *n*-hexane extract of the stems were isolated (−)-preorixine (**1**) and (+)-orixine (**2**), as well as (+)-isoptelefolidine (**3**) and several other quinoline alkaloids.[2−4)]

The stereochemistry at C-2′ of (+)-orixine (**2**) was reported by Bowman *et al.*[5)] as *R* by preparing the epoxide ($[\alpha]_D$ −0.85°) with the *S* configuration at the C-2′ position through semi-stereoselective synthesis using (+)-peroxycamphoric acid. They also stated that when the epoxide was treated with an acid the epoxide ring was opened at C-2′ with inversion to give the diols, and the configurations of other related alkaloids were estimated according to these results. However, the method of determination of the absolute configuration of the epoxide (**1**) obtained through synthesis involves uncertainty, namely, the asymmetric induction in the synthesis of **1** was only 2.4%[6)] and the absolute configuration of this compound was estimated only by the transition state model. In fact, the conclusion by Bowman *et al.* on the absolute stereochemistry of the C-2′ position of **1** and **2** in 1973[5)] was the opposite of their result reported in 1967.[6)] The isolation of naturally occurring (−)-epoxide (**1**, $[\alpha]_D$ −7.5°) prompted us to re-examine the configuration of the C-2′ position of this type of alkaloid.

When (−)-preorixine (**1**) was treated with dilute H_2SO_4 (H_2SO_4 : EtOAc = 1 drop : 20 ml), (+)-isoptelefolidine (**3**, 32% yield) was obtained along with (+)-orixine (**2**, 29% yield) and orixinone (**4**, 17% yield). The absolute configuration of (+)-isoptelefolidine (**3**), which must have the same absolute configuration at C-2′ position as does **1**, was studied by the extended Mosher method.[7,8)] Thus the alcohol (**3**) was transformed into its (*S*)-(−)- and (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters **5** and **6**, respectively. Their ^{1}H-NMR spectra were measured and the chemical shifts of the C-1′–C-5′ positions were compared.

Although these esters were partially racemized, the ^{1}H-NMR chemical shifts of the main components (**5A** and **6A**) were compared and the C-1′ methylene signals of (*S*)-Mosher ester (**5A**, δ 2.98 (1H, dd, *J* = 5, 14 Hz) and 3.26 (1H, dd, *J* = 9, 14 Hz)) appeared at lower field compared with those of (*R*)-Mosher ester (**6A**, δ 2.91 (1H, dd, *J* = 5, 14 Hz) and 3.24 (1H, dd, *J* = 9, 14 Hz)). On the other hand, the C-4′ methyl signal and the C-5′ terminal methylene signals of **5A** (δ 1.75 (3H, s) and 4.90, 4.94 (each 1H, br s)) appeared at higher field than those of **6A** (δ 1.88 (3H, s) and 4.97, 5.08 (each 1H, br s)). These results clearly showed that the configuration of the secondary alcohol at the C-2′ position of **3A** was *R*.

The absolute stereochemistry of (+)-orixine (**2**) obtained from the same plant material[2)] was also studied by preparing Mosher esters **7** and **8** and the results showed the C-2′ position of **2A** (main portion of **2**) to be *R* (Fig. 1). The results demonstrated that no inversion occurs during these reactions because (−)-preorixine (**1A**) can be transformed into (+)-orixine (**2A**) by treating **1A** with HCOOH followed by treating the formylated derivative (**9**) with dilute alkali.[2,5)] Though it was reported[5)] that **9** was obtained instead of **10** in this reaction, it is considered that when an epoxide is treated with HCOOH, an intermediate **10** is first formed and then transformed into **9** by acyl transfer (Fig. 2).

The secondary alcohols **2** and **3** were also obtained from the natural source,[3)] and the absolute configuration of the main portion of these natural compounds too was concluded to be *R* from these experiments.

In the ^{1}H-NMR of **1**, when a signal at δ 3.03 (1H, dd, *J* = 6, 9 Hz, H-2′) was irradiated, a 4% nuclear Overhauser effect (NOE) was observed at δ 1.31 (3H, s) (Fig. 3). On the other hand, when a signal at δ 7.51 (1H, d, *J* = 8 Hz, H-5) was irradiated, a 12% NOE was observed at one of the methoxyl signals at δ 3.98. Thus, the methyl signal at δ 1.31 and the methoxyl signal at δ 3.98 were assigned to Me-4′ and MeO-C_4, respectively, and all of the ^{1}H- and ^{13}C-NMR signals of **1** were assigned unambiguously.

The (*S*)-Mosher ester (**7**) of partially racemized (+)-orixine (**2**) was purified by repeated p-TLC using benzene–EtOAc (19 : 1) as solvent to afford **7A** and **7B**. The main component **7A** which was concluded to possess *R* configuration (C-2′) was hydrolyzed by methanolic alkali hydrolysis to give an optically pure (+)-orixine (**2A**) and the $[\alpha]_D$ value of **2A** was +77°. This result verifies that the C-2′ configuration of (+)-orixine (**2A**) is *R*.

In addition, (−)-preorixine (**1**) was reduced by treating it with B_2H_6/$LiBH_4$ to afford **11**. Then, compound **11** was acylated with (*R*)- and (*S*)-MTPA chloride to give **12** and **13**. Compounds **12** and **13** were partially racemized and ^{1}H-NMR signals of the main portion of each compound (**12A** and **13A**) were compared; it was found that, in the

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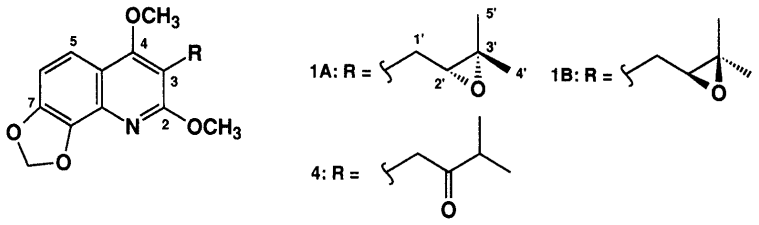
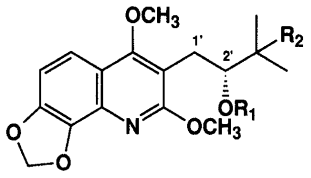
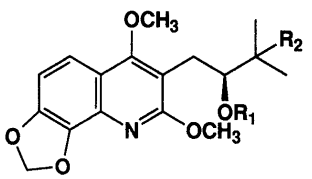
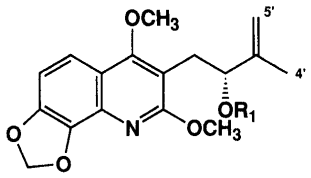




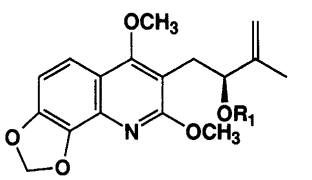
	R_1	R_2
2A:	H	OH
7A:	(*S*)-MTPA	OH
8A:	(*R*)-MTPA	OH
11A:	H	H
12A:	(*S*)-MTPA	H
13A:	(*R*)-MTPA	H
14A:	H	OCH_3
15A:	CH_3	OH
16A:	$COCH_3$	OCH_3
17A:	(*S*)-MTPA	OCH_3
18A:	(*R*)-MTPA	OCH_3



	R_1	R_2
2B:	H	OH
7B:	(*S*)-MTPA	OH
8B:	(*R*)-MTPA	OH
11B:	H	H
12B:	(*S*)-MTPA	H
13B:	(*R*)-MTPA	H
14B:	H	OCH_3
15B:	CH_3	OH
16B:	$COCH_3$	OCH_3
17B:	(*S*)-MTPA	OCH_3
18B:	(*R*)-MTPA	OCH_3



	R_1
3A:	H
5A:	(*S*)-MTPA
6A:	(*R*)-MTPA



	R_1
3B:	H
5B:	(*S*)-MTPA
6B:	(*R*)-MTPA

(*S*)-ester, C-1′ methylene signals (**12A**, δ 2.89 (1H, dd, $J=5$, 13.5 Hz), 3.17 (1H, dd, $J=9$, 13.5 Hz)) appeared at lower field compared with those of (*R*)-ester (**13A**, δ 2.81 (1H, dd, $J=5$, 13.5 Hz), 3.14 (1H, dd, $J=9$, 13.5 Hz)), while signals of the terminal dimethyl group (δ 0.91, 1.02 (each 3H, d, $J=7$ Hz)) in **12A** appeared at higher field than those (δ 1.05, 1.08 (each 3H, d, $J=7$ Hz)) of **13A**. These results showed that C-2′ positions of **12A** and **13A** were *R* and it was again verified that C-2′ position of **1A** was *R*.

A compound possessing a molecular formula $C_{18}H_{23}NO_6$ (MW 349) was obtained from the same extract from

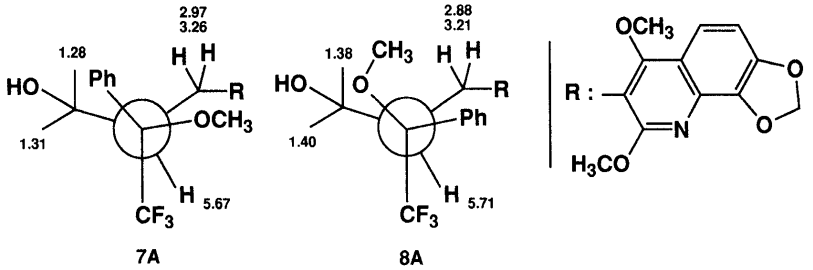


Fig. 1. Extended Newman Projection of (*S*)- and (*R*)-Mosher Esters (**7A** and **8A**) of (+)-Orixine (**2A**)

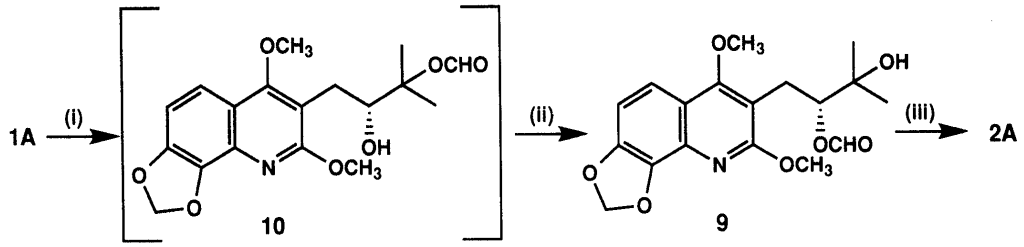


Fig. 2. Transformation of (−)-Preorixine (**1A**) into (+)-Orixine (**2A**)
(i) 90% HCOOH, r.t., 7 h; (ii) acyl transfer; (iii) aq. 2 N NaOH, r.t., 5 h.

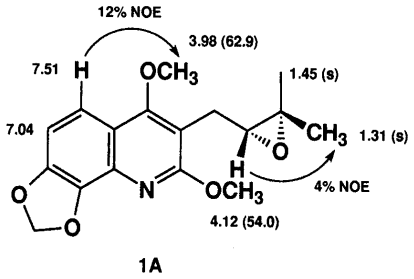


Fig. 3. NOE Experiment of (−)-Preorixine (**1A**)

which compounds **3** and **4** had been isolated. ^{1}H- and ^{13}C-NMR experiments on this compound showed that it possessed quite similar characteristics to those of (+)-orixine (**2**), except that this compound possessed an extra CH_3 (δ 3.29) group and structure of the compound was estimated to be either **14** or **15**. When this compound was treated with Ac_2O/pyridine, a monoacetyl derivative **16** was obtained. Because the methine signal of the mother compound (δ 3.80) was shifted to δ 5.40 in the acetylated compound, the structure of this acetate was deduced to be **16** and the position of the methoxyl group was concluded to be C-3′. From these observations, the structure of this compound was established as **14**, namely the 3′-*O*-methyl derivative of orixine (**2**) and the compound (main portion) was designated as (+)-3′-*O*-methylorixine (**14**). Because compound **14** could not be isolated from the *n*-hexane or from the acetone extract after *n*-hexane extraction, compound **14** might be an artifact derived from (−)-preorixine (**1**) during the isolation procedure using MeOH as solvent. In fact, when (−)-preorixine (**1**) was treated with MeOH under a mild acidic condition, compound **14** was obtained in good yield together with a small amount of orixinone (**4**) and a mixture of minor compounds. The occurrence of epoxide ring cleavage of **1** under an acidic condition at C-3′ position was also confirmed by this fact. Stereochemistry of **14** was studied by applying extended Mosher method after preparation of **17** and **18**, and it was concluded that the main portion of **14** (compound **14A**) possessed *R* configuration at C-2′ position.

Experimental

General Procedures UV spectra were measured with a Hitachi U-3200 spectrophotometer and IR spectra were recorded with a JASCO A-100S interferometer. EI-Mass spectra (EI-MS) were taken on a JEOL JMS-AX500 spectrometer. Optical rotations were measured on a JASCO DIP-370 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded with a Hitachi R-3000 or JEOL JNM GX-500 instrument using tetramethylsilane (TMS) as an internal standard and chemical shifts were recorded in δ units. Heteronuclear multiple-bond correlation (HMBC) spectra were measured at J=6 and 12 Hz on the JEOL JNM GX-500 spectrometer. Wakogel C-200 (Wako Pure Chemical Ind. Ltd.), ICN Alumina N, Akt. I (ICN Biomedicals), Cosmosil 75C_{18}-OPN (Nacalai Tesque Inc.) or Diaion HP-20 (Nippon Rensui Co.) were used for column chromatography (c.c.) and DC-Fertigplatten Kieselgel 60 F_{254} Art. 5715 (0.25 mm thick, Merck) was used for p-TLC. DC-Alufolien Kieselgel 60 F_{254} (0.2 mm thick, Merck) was used for TLC analyses.

Plant Material Plants of *O. japonica* THUNB. were collected near Sendai, Japan in May 1991. The plant was identified by one of us (S.F.) and a voucher specimen is deposited in the herbarium of the Dept. of Bioscience and Biotechnology, Aomori University.

Extraction and Isolation Air-dried stems (5.9 kg) of *Orixa japonica* were extracted with *n*-hexane (1 d, 3 times), MeOH (1 d, 3 times) and H_2O (2 d) at room temperature (r.t.) to afford 36 g, 556 g and 239 g of residue, respectively. Then, the MeOH ext. was further partitioned between $CHCl_3$–H_2O to afford $CHCl_3$ layer (128.1 g). (−)-Preorixine (**1**, 0.007%), (+)-orixine (**2**, 0.001%), (+)-isoptelefolidine (**3**, 0.002%) and orixinone (**4**, 0.017%) were isolated from the *n*-hexane ext. and **3** and **4** were also isolated from the $CHCl_3$ soluble layer of the MeOH ext. of this plant material.[2,3]

Isolation of (−)-Preorixine (1) Isolation procedure and UV, IR, EI-MS were reported previously.[2] ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.31 (3H, s, Me-4′), 1.45 (3H, s, Me-5′), 2.89 (1H, dd, J=9, 18 Hz, H-1′), 3.03 (1H, dd, J=7, 9 Hz, H-2′), 3.04 (1H, dd, J=7, 18 Hz, H-1′), 3.98 (3H, s, MeO-C_4), 4.12 (3H, s, MeO-C_2), 6.20, 6.21 (each 1H, d, J=3 Hz, –O–CH_2–O–), 7.05 (1H, d, J=8.5 Hz, H-6), 7.51 (1H, d, J=8.5 Hz, H-5); ^{13}C-NMR (125.65 MHz, $CDCl_3$) δ; 19.0 (q, Me-5′), 24.1 (t, C-1′), 24.9 (q, Me-4′), 54.0 (q, MeO-C_2), 59.2 (s, C-3′), 62.9 (q, MeO-C_4), 63.2 (d, C-2′), 102.2 (t, –O–CH_2–O–), 107.4 (d, C-6), 112.0 (s, C-3), 116.2 (d, C-5), 117:9 (s, C-4a), 132.7 (s, C-8a), 140.2 (s, C-8), 147.5 (s, C-7), 163.2 (s, C-2), 163.3 (s, C-4).

Isolation of (+)-3′-*O*-Methylorixine (14) Part of the $CHCl_3$ soluble layer (62.4 g) of the MeOH ext. of *O. japonica* stems was chromatographed over silica gel (1.25 kg) using $CHCl_3$ as solvent to give frs. 1—80 and then frs. 38—43 (8.05—10.20 l, 5.85 g) were further purified by silica gel c.c. (250 g) using *n*-hexane–EtOAc (7:4) as solvent and Cosmosil 75C_{18}-OPN c.c. (10 g) using MeOH–H_2O (2:1) as solvent followed by p-TLC using *n*-hexane–EtOAc (1:1) as solvent to give (+)-3′-*O*-methylorixine (**14**, 39.7 mg, 0.0014% yield) as a colorless oil; Rf=0.65 (*n*-hexane–EtOAc (1:1)); $[\alpha]_D^{23}$ +10.5° ($CHCl_3$, c=1.0); UV λ_{max} (MeOH) nm: 254 (ε_{max} 43600), 297 (3400), 316 (3400); IR ν_{max} (KBr) cm^{-1}: 3530, 2980, 2945, 1640, 1611, 1580, 1519, 1479, 1450, 1410, 1375, 1350, 1279, 1236, 1145, 1110, 1062, 1042; EI-MS *m/z* (rel. int. %): 349 (M^+, 20%), 277 (19), 262 (5), 246 (23), 232 (12), 216 (6), 201 (2); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.27 (6H, s, Me-4′, Me-5′), 2.54 (1H, br s, 2′-OH), 2.88 (2H, d, J=6 Hz, H-1′), 3.29 (3H, s, MeO-$C_{3'}$), 3.80 (1H, t, J=6 Hz, H-2′), 4.00 (3H, s, MeO-C_4), 4.10 (3H, s, MeO-C_2), 6.19 (2H, s, –O–CH_2–O–), 7.04 (1H, d, J=8 Hz, H-6), 7.49 (1H, d, J=8 Hz, H-5); ^{13}C-NMR (125.65 MHz, $CDCl_3$) δ: 19.9 (q, Me-5′), 20.7 (q, Me-4′), 26.4 (t, C-1′), 49.4 (q, MeO-$C_{3'}$), 54.0 (q, MeO-C_2), 62.5 (q, MeO-C_4), 76.0 (d, C-2′), 77.5 (s, C-3′), 102.2 (t, –O–CH_2–O–), 107.4 (d, C-6), 113.7 (s, C-3), 116.2 (d, C-5), 118.0 (s, C-4a), 132.5 (s, C-8a), 140.2 (s, C-8), 147.3 (s, C-7), 163.2 (s, C-2), 163.3 (C-4).

Treatment of (−)-Preorixine (1) with Dilute H_2SO_4 A solution of (−)-preorixine (**1**, 18.0 mg) in 1.5 ml conc. H_2SO_4/EtOAc (1 drop/20 ml) was stirred at r.t. for 21 h. The reaction mixture was partitioned with $CHCl_3$ (20 ml × 3) and H_2O (20 ml) and the organic layer was washed with H_2O (20 ml × 2) and concentrated *in vacuo*. The residue was applied to p-TLC (benzene–EtOAc (14:1)) to give (+)-orixine (**2**, 4.9 mg, 29% yield), (+)-isoptelefolidine (**3**, 5.4 mg, 32% yield), and orixinone (**4**, 2.9 mg, 17% yield).

Preparation of (+)-Isoptelefolidine-(*S*)-MTPA Ester (5A) (+)-Isoptelefolidine (**3**, 4.6 mg, 14.5 μmol), 4-dimethylaminopyridine (DMAP, 7.3 mg, 59.7 μmol, 4.1 eq) and triethylamine (TEA, 6.5 μl, 46.6 μmol 3.2 eq) were dissolved in 0.5 ml of dry CH_2Cl_2 and to this solution (*R*)-(−)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) Cl (10.8 μl, 57.7 μmol, 4.0 eq) was added, and the mixture was stirred at r.t. After 24 h, the reaction mixture was treated with *N*,*N*-dimethyl-1,3-propanediamine (7.2 μl, 57.2 μmol, 4.0 eq) for 15 min at r.t. The mixture was poured into H_2O (10 ml) and extracted with $CHCl_3$ (10 ml × 3). The combined $CHCl_3$ layers were evaporated *in vacuo* to give oily residue which was dissolved in a small amount of $CHCl_3$ and applied to p-TLC (benzene–EtOAc (19 : 1)) to afford a mixture of the diastereomers of isoptelefolidine (*S*)-MTPA ester (5.9 mg). The reaction product was applied to p-TLC (benzene–EtOAc (19 : 1)) to give (+)-isoptelefolidine (*S*)-MTPA ester (**5A**, 3.2 mg); *Rf* = 0.32 (benzene–EtOAc (19 : 1)); EI-MS *m/z*: 533 (M^+); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.75 (3H, s, Me-4′), 2.98 (1H, dd, *J* = 5, 14 Hz, H-1′), 3.26 (1H, dd, *J* = 9, 14 Hz, H-1′), 3.85 (3H, s, MeO-C_4), 4.09 (3H, s, MeO-C_2), 4.90, 4.94 (each 1H, br s, H-5′), 5.85 (1H, dd, *J* = 5, 9 Hz, H-2′), 6.22 (2H, s, –O–CH_2–O–), 7.05 (1H, d, *J* = 8.8 Hz, H-6), 7.46 (1H, d, *J* = 8.8 Hz, H-5). Compound **5B** (2.0 mg, *Rf* = 0.37 (benzene–EtOAc (19 : 1)) was obtained as a minor product.

Preparation of (+)-Isoptelefolidine-(*R*)-MTPA Ester (6A) In the same manner as for **5A**, (*R*)-MTPA ester of (+)-isoptelefolidine (**6A**, 4.2 mg) was obtained; *Rf* = 0.50 (benzene–EtOAc (19 : 1)); EI-MS *m/z*: 533 (M^+); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.88 (3H, s, Me-4′), 2.91 (1H, dd, *J* = 5, 14 Hz, H-1′), 3.24 (1H, dd, *J* = 9, 14 Hz, H-1′), 3.69 (3H, s, MeO-C_4), 4.10 (3H, s, MeO-C_2), 4.97, 5.08 (each 1H, br s, H-5′), 5.99 (1H, dd, *J* = 5, 9 Hz, H-2′), 6.21, 6.24 (each 1H, d, *J* = 1.5 Hz, –O–CH_2–O–), 7.01 (1H, d, *J* = 8.8 Hz, H-6), 7.31 (1H, d, *J* = 8.8 Hz, H-5). Compound **6B** (2.0 mg, *Rf* = 0.42 (benzene–EtOAc (19 : 1))) was obtained as a minor product.

Preparation of (+)-Orixine-(*S*)-MTPA Ester (7A) (+)-Orixine (**2**, 17.2 mg, 51.3 μmol), DMAP (19.4 mg, 158.8 μmol, 3.1 eq) and TEA (28.5 μl, 204.4 μmol, 4.0 eq) were dissolved in 1.0 ml of dry CH_2Cl_2 and to this solution (*R*)-(−)-MTPA Cl (36.2 μl, 204.1 μmol, 4.0 eq) was added, and the mixture was stirred at r.t. After 24 h, the reaction mixture was treated with *N*,*N*-dimethyl-1,3-propanediamine (25.4 μl, 201.9 μmol, 3.9 eq) for 15 min at r.t. The reaction mixture was poured into H_2O (20 ml) and extracted with $CHCl_3$ (20 ml × 3). The combined $CHCl_3$ layers were evaporated *in vacuo* to give oily residue which was dissolved in a small amount of $CHCl_3$ and applied to p-TLC (benzene–EtOAc (19 : 1)) repeatedly to afford (*S*)-MTPA ester of (+)-orixine (**7A**, 11.5 mg); *Rf* = 0.37 ; EI-MS *m/z*: 551 (M^+); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.28, 1.31 (each 3H, s, Me-4′, Me-5′), 2.97 (1H, dd, *J* = 3, 14 Hz, H-1′), 3.26 (1H, dd, *J* = 11, 14 Hz, H-1′), 3.84 (3H, s, MeO-C_4), 4.10 (3H, s, MeO-C_2), 5.67 (1H, dd, *J* = 3, 11 Hz, H-2′), 6.22 (2H, s, –O–CH_2–O–), 7.03 (1H, d, *J* = 8.4 Hz, H-6), 7.42 (1H, d, *J* = 8.4 Hz, H-5). Compound **7B** (1.1 mg, *Rf* = 0.42 (benzene–EtOAc (19 : 1))) was obtained as a minor product.

Preparation of (+)-Orixine-(*R*)-MTPA Ester (8A) In the same manner as for **7A**, (*R*)-MTPA ester of (+)-orixine (**8A**, 2.4 mg) was obtained; *Rf* = 0.43 (benzene–EtOAc (19 : 1)); EI-MS *m/z*: 551 (M^+); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.38, 1.40 (each 3H, s, Me-4′ and Me-5′), 2.88 (1H, dd, *J* = 3, 14 Hz, H-1′), 3.21 (1H, dd, *J* = 10, 14 Hz, H-1′), 3.65 (3H, s, MeO-C_4), 4.10 (3H, s, MeO-C_2), 5.71 (1H, dd, *J* = 3, 10 Hz, H-2′), 6.21, 6.25 (each 1H, s, –O–CH_2–O–), 7.00 (1H, d, *J* = 8.4 Hz, H-6), 7.25 (1H, d, *J* = 8.4 Hz, H-5). Compound **8B** (1.4 mg, *Rf* = 0.35 (benzene–EtOAc (19 : 1))) was obtained as a minor product.

Alkali Hydroxylation of (+)-Orixine-(*R*)-MTPA Ester (7A) A solution of the synthesized (*R*)-MTPA ester of (+)-orixine (**7A**, 9.9 mg) in 2 ml of MeOH/2 N KOH (3 : 1) was stirred at r.t. After 6 h, 2 ml of freshly prepared 2 N KOH was added to the reaction mixture which was further stirred for 2 d. The reaction mixture was poured into H_2O (20 ml) and extracted with $CHCl_3$ (20 ml × 3). The combined $CHCl_3$ layers were washed with H_2O (20 ml × 2) and dried over Na_2SO_4 (anhydrous) and evaporated *in vacuo*. The residue (4.9 mg) was applied to p-TLC (benzene–EtOAc (4 : 1)) to give enantiopure (+)-orixine (**2A**, 3.3 mg); $[\alpha]_D^{24}$ +77° ($CHCl_3$, *c* = 0.32).

Treatment of (−)-Preorixine (1) with B_2H_6/$LiBH_4$ A solution of (−)-preorixine (**1**, 25.2 mg, 79.5 μmol) in borane–THF (1.0 M solution in THF, 600 μl) containing large excess of $LiBH_4$ (19.4 mg) was stirred continuously at 0 °C. After 24 h, 0.5 M H_2SO_4–THF (1 : 1) was added at r.t., the reaction mixture was partitioned between $CHCl_3$ (20 ml) and H_2O (20 ml), the aqueous phase was extracted with $CHCl_3$ (20 ml × 2) and the combined $CHCl_3$ layers were washed with H_2O (20 ml × 2). Then, the $CHCl_3$ layer (60 ml) was dried over Na_2SO_4 (anhydrous) and evaporated *in vacuo* to give a brown residue (26.8 mg) which was applied to Si gel p-TLC using benzene–EtOAc (19 : 1) as developing solvent to afford 3′-dehydroxyorixine (**11**, 7.7 mg); $[\alpha]_D^{18}$ +11° ($CHCl_3$, *c* = 0.8); EI-MS *m/z*: 319 (M^+); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.03 (6H, d, *J* = 6.5 Hz, Me-4′, Me-5′), 1.77 (1H, d septet, *J* = 5, 6.5 Hz, H-3′), 2.79 (1H, dd, *J* = 9, 13.5 Hz, H-1′), 2.94 (1H, dd, *J* = 3, 13.5 Hz, H-1′), 3.70 (1H, ddd, *J* = 3, 5, 9 Hz, H-2′), 3.97 (3H, s, MeO-C_4), 4.10 (3H, s, MeO-C_2), 6.20 (2H, s, –O–CH_2–O–), 7.04 (1H, d, *J* = 8.4 Hz, H-6), 7.48 (1H, d, *J* = 8.4 Hz, H-5).

Preparation of (+)-3′-Dehydroxyorixine-(*S*)-MTPA Ester (12A) (+)-3′-Dehydroxyorixine (**11**, 1.7 mg, 5.3 μmol), DMAP (5.4 mg, 44.2 μmol, 8.3 eq) and TEA (4.5 μl, 32.3 μmol, 6.1 eq) were dissolved in 0.5 ml of dry CH_2Cl_2 and to this solution (*R*)-MTPA Cl (6.0 μl, 32.1 μmol, 6.1 eq) was added, and the mixture was stirred at r.t. After 24 h, the reaction mixture was treated with *N*,*N*-dimethyl-1,3-propanediamine (4.1 μl, 32.6 μmol, 6.1 eq) for 15 min at r.t., then poured into H_2O (10 ml) and extracted with $CHCl_3$ (10 ml × 3). The combined $CHCl_3$ layers were evaporated *in vacuo* to give an oily residue (7.2 mg) which was applied to p-TLC (benzene–EtOAc (9 : 1)) to afford the major (*S*)-MTPA ester of (+)-3′-dehydroxyorixine (**12A**, 1.3 mg); *Rf* = 0.37 benzene–EtOAc (9 : 1)); EI-MS *m/z*: 535 (M^+); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.91, 1.02 (each 3H, d, *J* = 7 Hz, Me-4′, Me-5′), 1.98 (1H, d septet, *J* = 4, 7 Hz, H-3′), 2.89 (1H, dd, *J* = 5, 13.5 Hz, H-1′), 3.17 (1H, dd, *J* = 9, 13.5 Hz, H-1′), 3.83 (3H, s, MeO-C_4), 4.09 (3H, s, MeO-C_2), 5.55 (1H, ddd, *J* = 4, 5, 9 Hz, H-2′), 6.21 (2H, s, –O–CH_2–O–), 7.04 (1H, d, *J* = 8.4 Hz, H-6), 7.45 (1H, d, *J* = 8.4 Hz, H-5). Compound **12B** (0.8 mg, *Rf* = 0.44 (benzene–EtOAc (9 : 1))) was obtained as a minor product.

Preparation of (+)-3′-Dehydroxyorixine-(*R*)-MTPA Ester (13A) In the same manner as for **12A**, the major moiety of (+)-3′-dehydroxyorixine-(*R*)-MTPA ester (**13A**, 1.3 mg) was obtained; *Rf* value = 0.43 (benzene–EtOAc (9 : 1)); EI-MS *m/z*: 535 (M^+); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.05, 1.08 (each 3H, d, *J* = 7 Hz, Me-4′, Me-5′), 2.03 (1H, d septet, *J* = 4, 7 Hz, H-3′), 2.81 (1H, dd, *J* = 5, 13.5 Hz, H-1′), 3.14 (1H, dd, *J* = 9, 13.5 Hz, H-1′), 3.71 (3H, s, MeO-C_4), 4.08 (3H, s, MeO-C_2), 5.62 (1H, ddd, *J* = 4, 5, 9 Hz, H-2′), 6.21, 6.23 (each 1H, d, *J* = 1.5 Hz, –O–CH_2–O–), 6.99 (1H, d, *J* = 8.4 Hz, H-6), 7.29 (1H, d, *J* = 8.4 Hz, H-5). Compound **13B** (0.8 mg, *Rf* = 0.37 (benzene–EtOAc (9 : 1))) was obtained as a minor product.

Preparation of (+)-3′-*O*-Methylorixine-(*S*)-MTPA Ester (17A) (+)-3′-*O*-Methylorixine (**14**, 2.9 mg, 8.30 μmol), DMAP (5.3 mg, 43.4 μmol, 5.2 eq) and TEA (5.0 μl, 35.9 μmol, 4.3 eq) were dissolved in 0.5 ml of dry CH_2Cl_2 and to this solution (*R*)-(−)-MTPA Cl (12.4 μl, 66.3 μmol, 4.9 eq) was added, and the mixture was stirred at r.t. After 24 h, the reaction mixture was treated with *N*,*N*-dimethyl-1,3-propanediamine (6.0 μl, 47.7 μmol, 5.7 eq) for 15 min at r.t., then poured into H_2O (10 ml) and extracted with $CHCl_3$ (10 ml × 3). The combined $CHCl_3$ layers were evaporated *in vacuo* to give an oily residue which was dissolved in a small amount of $CHCl_3$ and applied to p-TLC (Benzene–EtOAc (19 : 1)) repeatedly to afford (*S*)-MTPA ester of (+)-3′-*O*-methylorixine (**17A**, 2.6 mg); *Rf* = 0.43 (Benzene–EtOAc (19 : 1)); EI-MS *m/z*: 565 (M^+); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.22, 1.27 (each 3H, s, Me-4′, Me-5′), 2.96 (1H, dd, *J* = 3, 14 Hz, H-1′), 3.24 (1H, dd, *J* = 10.5, 14 Hz, H-1′), 3.28 (3H, s, MeO-$C_{3'}$), 3.83 (3H, s, MeO-C_4), 4.10 (3H, s, MeO-C_2), 5.90 (1H, dd, *J* = 3, 10.5 Hz, H-2′), 6.22 (2H, s, –O–CH_2–O–), 7.05 (1H, d, *J* = 8.4 Hz, H-6), 7.44 (1H, d, *J* = 8.4 Hz, H-5). Compound **17B** (2.2 mg, *Rf* = 0.50 (benzene–EtOAc (19 : 1))) was obtained as a minor product.

Preparation of (+)-3′-*O*-Methylorixine-(*R*)-MTPA Ester (18A) In the same manner as for **17A**, (*R*)-MTPA ester of (+)-orixine (**18A**, 2.6 mg) was obtained; *Rf* = 0.53 (benzene–EtOAc (19 : 1)); EI-MS *m/z*: 565 (M^+); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.33 (6H, s, Me-4′, Me-5′), 2.85 (1H, dd, *J* = 3, 14 Hz, H-1′), 3.19 (1H, dd, *J* = 10.5, 14 Hz, H-1′), 3.31 (3H, s, MeO-$C_{3'}$), 3.58 (3H, s, MeO-C_4), 4.10 (3H, s, MeO-C_2), 5.96 (1H, dd, *J* = 3, 10.5 Hz, H-2′), 6.22, 6.25 (each 1H, d, *J* = 1.5 Hz, –O–CH_2–O–), 6.98 (1H, d, *J* = 8.4 Hz, H-6), 7.24 (1H, d, *J* = 8.4 Hz, H-5). Compound **18B** (1.4 mg, *Rf* = 0.44 (benzene–EtOAc (19 : 1))) was obtained as a minor product.

Preparation of (+)-2′-*O*-Acetyl-3′-*O*-methylorixine (16) Ac_2O (0.05 ml) was added to the solution of (+)-3′-*O*-methylorixine (**14**, 3.8 mg) in pyridine (0.1 ml) and stirred under N_2 atmosphere at r.t. After 24 h, the solvent was evaporated *in vacuo* to afford a brown oil which was purified by p-TLC on Si gel using *n*-hexane–EtOAc (1 : 1) to give (+)-2′-*O*-acetyl-3′-*O*-methylorixine (**16**, 3.4 mg, 79.9% yield) as a colorless oil; *Rf* = 0.69 (*n*-hexane–EtOAc (1 : 1)); $[\alpha]_D^{28}$ +19° ($CHCl_3$,

$c=0.10$); UV λ_{max} (MeOH) nm: 254 (ε_{max} 44400), 317 (3300); IR ν_{max} (KBr) cm^{-1}: 2980, 2945, 1738, 1640, 1611, 1580, 1519, 1479, 1448, 1409, 1374, 1350, 1279, 1236, 1148, 1110, 1062, 1039; EI-MS *m/z* (rel. int. %): 391 (M^+, 11%), 331 (23), 316 (32), 300 (14), 276 (13), 260 (5), 246 (19), 232 (4), 216 (6); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.27, 1.28 (each 3H, s, Me-4′, Me-5′), 1.80 (3H, s, OCOMe), 2.93 (1H, dd, $J=3$, 14 Hz, H-1′), 3.12 (1H, dd, $J=11$, 14 Hz, H-1′), 3.34 (3H, s, MeO-$C_{3'}$), 3.97 (3H, s, MeO-C_4), 4.11 (3H, s, MeO-C_2), 5.40 (1H, dd, $J=3$, 11 Hz, H-2′), 6.18 (2H, s, –O–CH_2–O–), 7.02 (1H, d, $J=9$ Hz, H-6), 7.46 (1H, d, $J=9$ Hz, H-5).

Extraction of *O. japonica* Stems without Using MeOH Air-dried stems (20.1 g) of *O. japonica* were extracted with *n*-hexane (1 d × 2) followed by acetone (1 d × 3) to afford 39.6 mg and 351.5 mg of residue, respectively.

Treatment of (−)-Preorixine (1) with Acidic Methanol (−)-Preorixine (**1**, 5.2 mg) was dissolved in 1 ml of 12 N HCl/MeOH (1 : 100) solution and the solution was stirred at r.t. After 24 h, the reaction mixture was diluted with H_2O (20 ml) and extracted with $CHCl_3$ (20 ml × 3). The combined $CHCl_3$ layers were washed with H_2O (20 ml × 3) and dried over Na_2SO_4 (anhydrous) followed by concentration *in vacuo* to give a gummy residue (5.5 mg) which was purified by p-TLC (benzene–EtOAc (9 : 1)) to give 3′-*O*-methylorixine (**14**, 2.9 mg, $[\alpha]_D^{25}$ +9.0° ($CHCl_3$, $c=0.85$)), orixinone (**4**, 0.4 mg) and a mixture of minor compounds.

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