Pergamon 0031-9422-(95)00742-3 *Phytochemistry*, Vol. 41, No. 4, pp. 1231–1233, 1996

0031-9422/96 $15.00 + 0.00

(+)-3′-*O*-ACETYLISOPTELEFLORINE, A QUINOLINE ALKALOID FROM *ORIXA JAPONICA*

SHINJI FUNAYAMA,* KIYOSHI MURATA and SHIGEO NOZOE

Faculty of Pharmaceutical Sciences, Tohoku University, Aobayama, Sendai 980, Japan

(*Received* 11 *July* 1995)

Key Word Index—*Orixa japonica*; Rutaceae; stem; quinoline alkaloid; (+)-3′-*O*-acetylisopteleflorine.

Abstract—A new quinoline alkaloid, (+)-3′-*O*-acetylisopteleflorine, was isolated from a methanol extract of the stems of *Orixa japonica*. Its structure was elucidated by a combination of NMR analyses and chemical reactions.

INTRODUCTION

Orixa japonica Thunb. 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. Previously, several new quinoline alkaloids have been obtained from extracts of this species [1–3]. In the present paper we report the isolation and structural elucidation of a new quinoline alkaloid, (+)-3′-*O*-acetylisopteleflorine (1), isolated from a methanol extract of the stems of *O. japonica*.

RESULTS AND DISCUSSION

The UV spectrum of compound **1**, λ_{max} (MeOH) 254 and 313 nm, suggested a quinoline or quinolone moiety; it was concluded to be a quinoline because the ε_{max} of the latter absorption maximum was small (3700) [3]. In the ^{13}C NMR spectrum of **1**, 18 signals, including four methyl, two methylene, three methine and nine quaternary carbons were observed (Table 1) and in the EI-mass spectrum, the $[M]^+$ appeared at *m/z* 345. Thus, the molecular formula of this compound was estimated to be $C_{18}H_{19}NO_6$, which was subsequently established by HREI-mass spectrometry (observed 345.1208; calculated 345.1213). In the ^{1}H NMR spectrum of **1** (Table 1), a methyl signal attributed to an acetyl moiety (δ1.97) was observed, along with a geminal methyl (δ1.55 and δ1.62), methoxyl (δ4.22), methylenedioxy (δ6.15 (2H)), three sp^3 signals coupled to each other (δ3.49, δ3.62 and δ4.97) and two coupled aromatic signals (δ6.95 and δ7.58). The existence of an acetyl moiety was also proved by the existence of an ion at *m/z* 303 $[M - 42]^+$ in the EI-mass spectrum and a band at 1737 cm^{-1} in the IR spectrum. Together, these results indicated that this quinoline alkaloid possessed a dihydrofuran or dihydropyran moiety and the structure of this compound was deduced as one of the isomers **1**–**4**. Among these, compound **2** is attributed to the monoacetyl derivative of pteleflorine (**5**) [4].

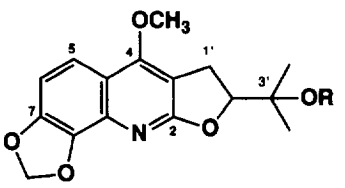
Table 1. ^{1}H and ^{13}C NMR assignments of (+)-3′-*O*-acetylisopteleflorine (**1**)

C	δ_C	δ_H
2	169.5 *s*	—
3	99.7 *s*	—
4	159.3 *s*	—
4*a*	116.7 *s*	—
5	116.1 *d*	7.58 *d* (9)*
6	106.7 *d*	6.95 *d* (9)
7	147.9 *s*	—
8	140.2 *s*	—
8a	133.9 *s*	—
1′	29.0 *t*	3.49 *dd* (7,16)
		3.62 *dd* (9,16)
2′	84.1 *d*	4.97 *dd* (7,9)
3′	82.2 *s*	—
4′ (3′-CH_3)	20.9 *q*	1.55 3H, *s*
5′ (3′-CH_3)	22.4 *q*	1.62 3H, *s*
C_4-OCH_3	58.4 *q*	4.22 3H, *s*
–OCH_2O–	102.2 *t*	6.15 2H, *d* (each 2)
$COCH_3$	22.4 *q*	1.97 3H, *s*
$\underline{C}OCH_3$	170.3 *s*	—

*Figures in parentheses are coupling constants (Hz).

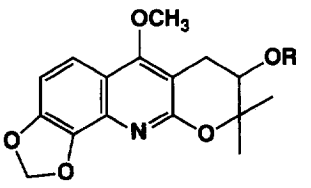
In the long-range ^{13}C–^{1}H COSY spectrum, δ_C159.3, assigned to C_4, was coupled to δ_H3.49 (H-1′), 4.22 (OCH_3) and 7.58 (H-5) (Fig. 1). Consequently, the position of a methylenedioxy moiety (between C-7 and C-8) was confirmed and the angular isomers **3** and **4** were excluded as the strcture of this compound. Conversely, when δ_H4.22 (C-4, OCH_3) was irradiated, a 1% NOE was observed for δ_H7.58 (H-5) and 2% and 3% NOE effects were observed for δ_H3.62 and 3.49 (H_2-1′), respectively.

*Author to whom correspondence should be addressed.



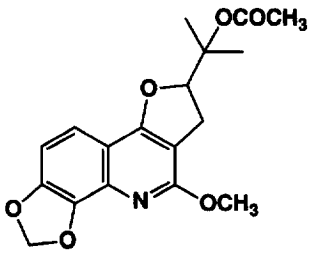
1 R = $COCH_3$

6 R = H

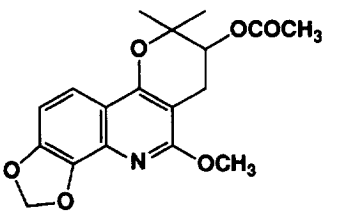


2 R = $COCH_3$

5 R = H

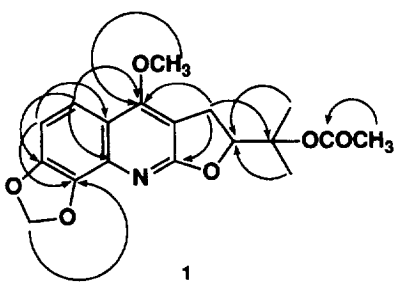


3



4

These observations also supported the linear system of this compound.



1

Fig. 1. ^{13}C–^{1}H correlations in the long-range C–H COSY spectrum of 3′-*O*-acetylisopteleflorine (**1**).

Compound **1** was hydrolyzed with dilute alkali to give an alcohol **6** ($C_{16}H_{17}NO_5$, M_r 303). In the ^{1}H NMR spectrum of **6** almost all of the signals appeared similar to the parent compound, except for the absence of the methyl signal of the acetyl moiety of **1**. The oxygen-bearing methine signal (δ4.64) in **6** appeared at lower field compared with that of C-2′-H (δ_H3.74) in pteleflorine (**5**) [3] and was not changed dramatically compared with that of **1** (δ4.97). Consequently, the acetyl moiety was not attached to the oxygen next to the methine moiety. In addition, when the alcohol **6** was treated with Ac_2O/pyridine, no acetylation occurred and, thus, the alcohol moiety was established as tertiary. From these observations the structure was concluded to be a linear furanoquinoline. Assignments of the ^{1}H NMR and attached ^{13}C NMR signals were achieved by a ^{13}C–^{1}H COSY spectrum and by comparison with existing data [3]. Unambiguous assignments of the quaternary carbons of **1** were accomplished using the long-range ^{13}C–^{1}H COSY spectral data, as shown in Fig. 1 and Table 1.

Compound **6** has been synthesized previously as a by-product in the synthesis of pteleflorine (**5**) [4], but **1** has not been reported previously. Compounds **6** and **1** were designated as (−)-isopteleflorine and (+)-3′-*O*-acetylisopteleflorine, respectively. Until now, *O*-methylbalfourodinium salt (as a perchlorate) is the only other alkaloid isolated from *O. japonica* [5] possessing a dihydrofuran moiety.

EXPERIMENTAL

General. ^{1}H NMR and ^{13}C NMR were recorded on a JEOL JNM GX-500 or HITACHI R-3000 instrument; TMS was used as int. standard and chemical shifts are recorded in δ units. Wacogel C-200 (Wako Pure Chemical Ind., Ltd.) and Cosmosil 75C_{18}-OPN (Nacalai Tesque, Inc.) were used for CC and DC-Fertigplatten Kieselgel 60 F_{254} (Merck) for prep. TLC.

Plant material. O. japonica plants were collected near Sendai, Japan in May, 1991. The plant was identified by S.F. and a voucher specimen is deposited in the herbarium of this department.

Extraction and isolation. Air-dried stems (5.9 kg) were extracted with *n*-hexane at room temp. (3×) to yield a residue (36 g), which was further extraced with MeOH to yield 556 g. The MeOH extract was partitioned between $CHCl_3$–H_2O to yield $CHCl_3$-solubles (128.1 g). Part of this fr. (62.4 g) was chromatographed over silica gel (1.25 kg) using $CHCl_3$ to give 80 frs. Frs. 38–43 (8.05–10.20 l, 5.85 g) were further purified by silica gel CC (250 g) using *n*-hexane–EtOAc (7:4) and Cosmosil 75C_{18}-OPN CC (10 g) using MeOH–H_2O (2:1) followed by prep. TLC using *n*-hexane–EtOAc (1:1), to give (+)-3′-*O*-acetylisopteleflorine (**1**) (R_f = 0.47, 56.1 mg, 0.002% yield).

(+)-3′-*O-Acetylisopteleflorine* (**1**). Oil. UV λ_{max} (MeOH): 254 (ε_{max} 39300), 313 (3700) nm. IR ν_{max} (KBr): 2990, 1737, 1638, 1539, 1480, 1420, 1274, 1111, 1041, 980 cm^{-1}. EI-MS *m/z* (rel. int.): 345 ($[M]^+$, 50), 303 (4), 285 (49), 270 (100), 255 (12), 244 (33), 232 (10), 202 (7), 186 (6), 135 (5). HREI-MS: obsd *m/z* 345.1208, calcd

for $C_{18}H_{19}NO_6$ 345.1213. $[\alpha]_D^{25}$ +6.4° ($CHCl_3$, *c* 0.7). ^1H and ^{13}C NMR ($CDCl_3$); see Table 1.

Hydrolysis of (+)-3′-*O*-*acetylisopteleflorine* (**1**). To (+)-3′-*O*-acetylisopteleflorine (**1**) (15 mg) in MeOH (3 ml) was added aq. 2 N KOH (1 ml) and the mixt. stirred at room temp. After 26 hr, the reaction mixt. was dil. with H_2O (20 ml), and extracted with $CHCl_3$ (3 × 20 ml). The combined $CHCl_3$ layers were washed with H_2O (3 × 20 ml), dried (Na_2SO_4) and evapd *in vacuo* to provide a gummy residue (12.9 mg) which was purified by prep. TLC using *n*-hexane–EtOAc (1:1) to give (−)-isopteleflorine (**6**) (10.9 mg, 83% yield).

(−)-*Isopteleflorine* (**6**). Oil. UV λ_{max} (MeOH): 255 (ε_{max} 40 400), 314 (3900) nm. IR ν_{max} (KBr): 3350, 2940, 1636, 1593, 1539, 1481, 1420, 1367, 1350, 1271, 1110, 1042, 981 cm^{-1}. EI-MS *m/z* (rel. int.): 303 ($[M]^+$, 82), 285 (11), 270 (25), 244 (100), 231 (17), 217 (24), 202 (24), 186 (15), 174 (10), 156 (6), 149 (16), 116 (11), 103 (8). $[\alpha]_D^{21}$ − 15.2° ($CHCl_3$, *c* 0.2). ^1H NMR ($CDCl_3$): δ1.26 and δ1.42 (each 3H, *s*, C-3′-CH_3 × 2), 3.54 (1H, *dd*, *J* = 17, 8 Hz, H-1′), 3.59 (1H, *dd*, *J* = 17, 9 Hz, H-1′), 4.23 (3H, *s*, C-4–OCH_3), 4.63 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 6.15 (2H, each 1H, *d*, *J* = 2 Hz, –OCH_2O–), 6.95 (1H, *d*, *J* = 9 Hz, H-6), 7.60 (1H, *d*, *J* = 9 Hz, H-5).

Acetylation of (−)-*isopteleflorine* (**6**). Compound **6** (2.2 mg) was dissolved in pyridine (0.5 ml), Ac_2O (0.25 ml) added and the soln stirred under a N_2 atmosphere for 24 hr. The reaction mixt. was dil. with H_2O (20 ml) and extracted with $CHCl_3$ (3 × 20 ml). The combined $CHCl_3$ layers were washed with H_2O (3 × 20 ml), dried (Na_2SO_4) and concd *in vacuo* to give unreacted **6** (1.8 mg).

Acknowledgements—We thank the analytical centre of this institute for NMR and MS data. The authors also thank Messrs Hideki Hayasaka and Keiji Ohba of the Medicinal Plant Garden of Tohoku University for collecting the plant material.

REFERENCES

1. Terasaka, M. (1931) *Yakugaku Zasshi* **51**, 707.
2. Funayama, S., Kageyama, T., Murata, K., Adachi, M. and Nozoe, S. (1993) *Heterocycles* **35**, 607.
3. Funayama, S., Murata, K. and Nozoe, S. (1994) *Phytochemistry* **36**, 525.
4. Neville, C. F., Grundon, M. F., Ramachandran, V. N. and Reisch, J. (1991) *J. Chem. Soc. Perkin Trans. I* 259.
5. Donnelly, W. J. and Grundon, M. F. (1972) *J. Chem. Soc. Perkin Trans. I* 2116.