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IMPERANENE, A NOVEL PHENOLIC COMPOUND WITH PLATELET AGGREGATION INHIBITORY ACTIVITY FROM *IMPERATA CYLINDRICA*

KIMIHIRO MATSUNAGA, MASAOKI SHIBUYA,[1] and YASUSHI OHIZUMI*

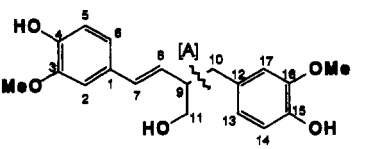
Department of Pharmaceutical Molecular Biology, Pharmaceutical Institute, Tohoku University, Aoba, Aramaki, Aoba-ku, Sendai 980, Japan

ABSTRACT.—Imperanene, a novel phenolic compound [**1**] has been isolated from *Imperata cylindrica*. Its structure was elucidated by spectroscopic evidence. Imperanene showed platelet aggregation inhibitory activity.

Numerous compounds with platelet aggregation inhibitory activity have been employed in basic physiological research. In our search for platelet aggregation inhibitory substances from medicinal plants, imperanene [**1**] has been isolated as an active substance from *Imperata cylindrica* (L.) Beauv. (Gramineae) (Japanese name "Chigaya"). In this communication, we report the isolation and structure elucidation of imperanene and its activity.

The rhizomes of *I. cylindrica* have been used in Chinese medicine as diuretic and anti-inflammatory agents (1-3). However, only a few studies concerning the chemical constituents of this plant have been reported (4-7).

The EtOAc solubles of the MeOH and H_2O extracts of rhizomes of *I. cylindrica* were repeatedly chromatographed over Si gel to afford imperanene [**1**]. This newly isolated compound **1**, $[\alpha]^{25}D$ +700° (c=1.0, $CHCl_3$), showed a molecular ion at m/z 330 in the eims. The ir spectrum of **1** displayed absorption bands at 3400 and 1600 cm^{-1}, indicating the presence of hydroxyl and olefinic functionalities. In the ^{1}H-nmr spectrum of **1**, there were two olefin protons at δ 6.33 (1H, d, J=16.0 Hz) and δ 5.90 (1H, dd, J= 16.0 and 8.4 Hz). These spectral features are characteristic for an *E*-olefin conjugated with a



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phenyl ring. Detailed analysis of the ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY spectra of **1** permitted assignments of all the ^{1}H- and ^{13}C-nmr signals as follows: ^{1}H nmr ($CDCl_3$, 500 MHz) δ 2.62 (1H, m, H-9), 2.70 (2H, complex, H_2-10), 3.53 (1H, dd, J=10.7 and 7.2 Hz, H-11), 3.65 (1H, dd, J=10.7 and 5.0 Hz, H-11), 3.81 (3H, s, OMe), 3.87 (3H, s, OMe), 5.46 (1H, s, OH), 5.62 (1H, s, OH), 5.90 (1H, dd, J= 16.0 and 8.4 Hz, H-8), 6.33 (1H, d, J=16.0 Hz, H-7), 6.66 (2H, complex, Ar-H), 6.80 (1H, d, J=8.8 Hz, Ar-H), 6.83 (3H, complex, Ar-H); ^{13}C nmr ($CDCl_3$, 125 MHz) δ 37.7 (t, C-10), 47.7 (d, C-9), 55.9 (q, OMe), 56.0 (q, OMe), 65.3 (t, C-11), 108 (d, C-6), 112 (d, C-13), 114.2 (d, C-5), 114.5 (d, C-14), 120 (d, C-2), 122 (d, C-17) 128 (d, C-8), 130 (s, C-1), 132 (s, C-12), 132.4 (d, C-7), 144 (s, C-15), 145 (s, C-4), 146 (s, C-3), 147 (s, C-16). From the results of the COLOC spectrum, the structure of imperanene was determined as shown. In the eims spectrum, the fragments at m/z 137 and 193 can be accounted for via pathway [A].

Imperanene [**1**] gave complete inhibition at 6×10^{-4} M against rabbit platelet aggregation induced by thrombin (0.5 units), but did not affect the enzyme

[1]Research Laboratories Torii & Co., Ltd., 1-2-1 Ohnodai, Midori-ku, Chiba, 267, Japan.

activities of cyclic AMP phosphodiesterase, Na^+-K^+ ATPase, tyrosinase and 5-lipoxygenase, nor the functions of the sarcoplasmic reticulum. Any other physiological functions of this class of compounds would be of interest for future investigation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The optical rotation was measured on a Jasco DIP-360 digital polarimeter. The uv spectrum was taken on a Hitachi U-2000 spectrometer. The ir spectrum was taken on a Shimadzu IR-408 spectrometer. The eims spectrum was obtained on a JEOL JMS AX-500 spectrometer. ^1H- and ^{13}C-nmr spectra were recorded on a JEOL JNM GX-500 spectrometer using TMS as internal standard.

PLANT MATERIAL.—The rhizomes of *I. cylindrica* were supplied by Nippon Hunmatsu Yakuhin, Ltd., Osaka, Japan. A voucher specimen has been deposited in the herbarium department of this facility.

EXTRACTION AND ISOLATION.—A 2-kg quantity of the rhizomes of *I. cylindrica* was extracted with boiling MeOH (2 liters×3) and H_2O (2 liters×3), to give an extract which was partitioned with EtOAc and H_2O. The EtOAc solubles were subjected to Si gel cc (Kieselgel 60, Merck) eluted with $CHCl_3$-MeOH (100:1) to give a pale yellow oil. The fraction was repeatedly separated by Si gel tlc [solvents: $CHCl_3$-Me_2CO (10:1)], C_6H_6-MeOH (7:1), and $CHCl_3$-EtOAc (1:1) to give imperanene [**1**] (8 mg) as a colorless oil: $[\alpha]^{25}D$ +700° (c=1.0, $CHCl_3$); uv (MeOH) λ max 270 (ε 9174) nm; eims m/z $[M]^+$ 330 (32), 193 (86), 175 (86), 137 (100); hrms m/z found 330.1479, calcd 330.1491 for $C_{19}H_{22}O_5$; ir (film) ν max 3400, 1600 cm^{-1}; ^1H- and ^{13}C-nmr data, see text.

BIOASSAYS.—These were based on a platelet aggregation procedure published by Watanabe *et al.* (8). Fresh blood was obtained from male Japanese white rabbits and subsequently centrifuged at 250×g for 10 min to obtain platelet-rich plasma (PRP). The PRP was centrifuged at 180×g for 5 min. The platelet fraction was washed twice by suspension and centrifugation (180×g, 5 min) with tyrode-HEPES-albumin solution (pH 6.35). The resultant pellet was resuspended in the tyrode-HEPES-albumin solution (pH 7.35) with a final density of approximately 2×10^8 cells/ml. The tyrode-HEPES-albumin solution was composed of NaCl 138 mM, KCl 2.68 mM, $MgCl_2\cdot6H_2O$ 1.05 mM, $NaHCO_3$ 4.0 mM, HEPES 10 mM, glucose 0.1%, bovine serum albumin 0.35%. A 0.5 ml of platelet pellet prepared as described above was preincubated for 5 min at 37°, followed by the addition of 5 μl of 100 mM $CaCl_2$ and further preincubation for 5 min. Then, 5 μl of imperanene [**1**] (6×10^{-2} M solution) were added, and, after 5 min, 5 μl of thrombin (50 units solution) were added.

The enzymatic activities of cyclic AMP phosphodiesterase (9), Na^+-K^+ ATPase (10), tyrosinase (11) and 5-lipoxygenase (12) were measured, and the Ca^{2-} electrode experiments for sarcoplasmic reticulum functions (13) were performed as previously reported.

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