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Anti-arrhythmia Constituent in *Herba Leonuri*

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The 70% aqueous acetone extract of *Herba Leonuri* has shown anti-arrhythmic activity by the screening of digoxin-induced arrhythmia using papillary muscle of guinea-pig. On the investigation of the active compound by the activity directed fractionation, stigmast-4-en-3-one (β-sitostenone) was isolated. The ED_{50} of anti-arrhythmic activity of β-sitostenone was 35 μg/ml.

Keywords: *Herba Leonuri*, *Leonurus japonicus* Houtt., aerial parts, Anti-arrhythmia, β-sitostenone, stigmasta-4-en-3-one

Chinese herbal medicine *Herba Leonuri* (Yakumo-sou, 益母草) is the aerial part of *Leonurus japonicus* Houtt. (*Labiatae*) collected during flowering[1]. This crude drug had been used for irregular menstruation, severe cramps and other symptoms of women, in Japan[2,3].

On the constituents of *Herba Leonuri*, alkaloids, leonurine and stachydrine were isolated along with steroids, diterpenes, vitamin A, and oleinic acid[4-5].

In the course of our studies on anti-arrhythmia constituents in crude drugs, the 70% aqueous acetone extract of *Herba Leonuri* has shown to be active by the screening of digoxin-induced arrhythmia using papillary muscle of guinea-pig[6-8]. In this paper, we report the isolation and characterization of anti-arrhythmia constituents in *Herba Leonuri*.

RESULT and DISCUSSION

The aqueous acetone extract of *Herba Leonuri* was suspended in water and was partitioned with ether, ethyl acetate, chloroform and *n*-butanol, successively. Among these fractions, the ether soluble fraction showed the strongest anti-arrhythmic activity. The activity directed fractionation of the ether soluble fraction was performed by chromatography using silica gel, reversed phase silica gel. Finally, the active compound (**1**) was isolated by preparative HPLC.

Compound **1**, obtained as a white needle from methanol, showed molecular ion peak at *m/z* 412, and the molecular formula was determined as $C_{29}H_{48}O$ by HR-EI-MS. In the ^{1}H-NMR spectrum, compound **1** showed characteristic methyl signals, which implied the sterol skeleton. The additional signal of olefinic proton was observed at δ 5.71, which must be assigned as the α proton of α, β-unsaturated ketone. This assumption was supported by the ^{13}C-NMR spectrum of **1**. The carbonyl carbon was observed at δ 199.7, and the olefinic carbons were observed at δ 123.7 and 171.8. By comparison of the spectral data of **1** with those of the literature[9-10], compound **1** was characterized as stigmasta-4-en-3-one (β-sitostenone).

The ED_{50} of anti-arrhythmic activity of **1** was 35 μg/ml, and this value is about one fourth of the ether extract and one fifth of the starting extract. (Table 1) The ED_{50} value of disopyramide, a commercially available anti-arrhythmic drug, was shown to be 17 μg/ml. Furthermore, synthesized **1** from commercial β-sitosterol also gave a comparable result.

Therefore, compound **1** may concern the anti-

arrhythmic activity of *Herba Leonuri*.

In addition, other plant steroid derivatives (stigmast-4,22-dien-3-one, etc.) were also isolated and examined anti-arrhythmic activity. Surprisingly, these compounds have not shown anti-arrhythmic activity at all, despite their structural similarity. The detailed result will be reported in the full paper.

Table 1. Anti-arrhythmic activity of compound **1** and extracts.

Concentration (mg/ml)	ED_{50} *(μg/ml)
compound **1**	35
70% acetone ext.	170
Ether layer	130
Ethyl acetate layer	>1000
chloroform layer	>3000
n-butanol layer	>1000
aqueous layer	>3000

*n=3

EXPERIMENTAL

Melting points were determined by using a Yanagimoto micro melting point apparatus and were uncorrected. EI and SI-MS spectra were obtained with a JEOL JMS-FABmate mass spectrometer, Optical rotation were measured using a JASCO DIP-360 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JMN-LA300. Silica-gel 60 (Merck 0.063-0.200mm) and ODS silica-gel (Fuji-Silysia DM1020T) was used for column chromatography. Shimadzu LC-10 apparatus and Kanto-Kagaku Mightysil RP-18 column were used for preparative HPLC.

Material

Herba Leonuri was purchased from Chinese Market. It was harvested near Beijing while flowering.

Extraction and Isolation

Herba Leonuri (1.0kg) was extracted with 70% aqueous acetone at room temperature and the extract was evaporated. The residue (47.8g) was suspended in water and was partitioned with ether, ethyl acetate, chloroform and *n*-butanol. Ether extract (10.3g) was separated into two fractions by silica gel column chromatography using n-Hexane-EtOAc (5:1) and $CHCl_3$-MeOH (5:1). The former fraction (4.6g) was chromatographed on ODS silica-gel eluting with MeOH-H_2O (9:1) and then $CHCl_3$. $CHCl_3$ fraction (1.0g) was subjected to silica-gel column chromatography eluting with increasing amount of EtOAc in n-Hexane and $CHCl_3$-MeOH (9:1) to give four fractions. Preparative HPLC (column: Mightysil RP-18) of the second fraction (116.6mg) with MeOH-H_2O (98:2) gave compound **1** (54.1mg).

Anti-arrhythmic Activities

Field stimulation (2Hz, 10V) was given to papillary muscle of guinea pig in Krebs-Henseleit buffer. After rhythmic contraction was observed, arrhythmia was artificially induced by applying digoxin (1μM). The buffer was changed to that containing test sample and digoxin. When test sample was insoluble in the buffer, methanol (max. 1%, which concentration has no effect on anti-arrhythmic activity) was added as a solubilizer. If the anti-arrhythmic effect was observed and the effect continued even after changing to the buffer containing only digoxin, the sample was concluded to be effective.

Stigmasta-4-en-3-one (**1**, β-sitostenone)

Colorless needles from methanol, mp 95-96.5℃. $[\alpha]_D^{25}$ +68.6° (c =0.22, $CHCl_3$). EI-MS *m/z*: 412 $[M]^+$, 397, 370, 355, 389, 229, 124. HR-EI-MS *m/z*: 412.3699 (Calcd for $C_{29}H_{48}O$: 412.3705). ^{1}H-NMR ($CDCl_3$) δ: 5.71 (1H, s, 4-H) ^{13}C-NMR ($CDCl_3$) δ: 35.66(C-1), 33.96(C-2), 199.69(C-3), 123.71(C-4), 171.75(C-5), 32.94(C-6), 32.03(C-7), 35.60(C-8), 53.79(C-9), 38.58(C-10), 21.01(C-11), 39.60(C-12), 42.37(C-13), 55.85(C-14), 24.16(C-15), 28.18(C-16), 55.98(C-17), 11.93(C-18), 17.36(C-19), 36.10(C-20), 18.68(C-21), 33.85(C-22), 26.07(C-23), 45.80(C-24), 29.12(C-25), 19.80(C-26), 19.01(C27), 23.04(C-28), 11.96(C-28).

Synthesis of β-sitostenone

Synthetic β-sitostenone derived from the commercial available β-sitosterol *via* two steps (PCC oxidation and isomerization of double bond with *p*-toluene sulfonic acid) described as follows; β-sitosterol (200 mg) was treated with PCC (200 mg) in anhydrous CH_2Cl_2 (10 ml) for 4 hours at room temperature. After silica-gel column chromatography of the resulting mixture using n-Hexane-EtOAc (10:1) as an eluent and evaporation of the solvent in vacuo, the remaining residual solid was treated

with *p*-toluenesulphonic acid (140 mg) in CH_2Cl_2 (5 ml) for 20 minutes at room temperature. After removal of the solvent in vacuo, the residual solid was purified on reversed phase HPLC (column: Mightysil RP-18) using MeOH-H_2O (98:2) as an eluent to give the β–sitostenone (88 mg). All spectral data corresponded with that of the compound **1**.

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