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Studies on Bioactive Substances in Crude Drugs Used for Arthritic Diseases in Traditional Chinese Medicine. III.[1] Isolation and Identification of Anti-inflammatory and Analgesic Principles from the Whole Herb of *Pyrola rotundifolia* L.

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Anti-inflammatory and analgesic principles were isolated from the methanol extract of the whole herb of *Pyrola rotundifolia* L., based on bioassays of the inhibitory activities on carrageenan-induced hind paw edema in rats and on acetic acid-induced writhing in mice. The principles were identified as ursolic acid and chimaphilin.

Keywords——anti-inflammatory principle; analgesic principle; *Pyrola rotundifolia*; ursolic acid; chimaphilin; anti-arthritic principle

In the last few years, we have been attempting to isolate anti-inflammatory and analgesic principles from a group of Chinese crude drugs called Qu-feng-shi-yao (祛風湿薬), which has long been used as a remedy mostly for arthritic diseases in traditional Chinese medicine. This report deals with anti-inflammatory and analgesic principles in the methanol extract of Pyrolae Herba.

Pyrolae Herba ("Lu-ti-cao" in Chinese), the whole herb of *Pyrola rotundifolia* L., has been used as a remedy mainly for arthritic diseases, gastric hemorrhage and pulmonary hemorrhage.[2]

Anti-inflammatory and analgesic tests were carried out by using carrageenan-induced hind paw edema in rats and acetic acid-induced writhing in mice, respectively. Active compounds I and II in the methanol extract of the herb were isolated as described in Experimental. Table I shows the biological activities and yields of each fraction from Pyrolae Herba (1 kg).

The active compounds I and II were identified as chimaphilin and ursolic acid, respectively, by direct comparison of their physical properties with those of authentic samples. Chimaphilin showed significant inhibitory activities on both edema (33% inhibition at a dose of 500 mg/kg, $p<0.05$) and writhing (49% inhibition at a dose of 150 mg/kg, $p<0.01$) even on oral administration. Ursolic acid also showed significant inhibitory activities on both edema (24% inhibition at a dose of 500 mg/kg, $p<0.05$) and writhing (31% inhibition at a dose of 150 mg/kg, $p<0.01$) on oral administration.

Past work on the whole herb of *Pyrola rotundifolia* L. has demonstrated the occurrence of a variety of compounds.[3] However, no one has previously correlated the constituents to the anti-inflammatory and analgesic activities. This is thus the first report on the biological action of the constituents of the whole herb of *Pyrola rotundifolia* L.

TABLE I. Biological Activities of Fractions from Pyrolae Herba (1 kg)

Fractions and compounds	Yield (g)	Inhibitory activity (Inhibition %/dose, mg/kg)	
		Anti-inflammatory[a] (*i.p.*)	Analgesic[b] (*s.c.*)
MeOH ext.	104	46/250[c]	68/1000[c]
n-BuOH sol.	65.8	41/200[c]	69/ 500[c]
H_2O sol.	38.2	—/200	25/ 500
Ether ext.	31.4	33/100[c]	66/ 250[c]
Residue	34.4	—/100	—/ 250
Fraction 1	3.4	23/ 50	27/ 50
Fraction 2	1.8	35/ 50[c]	44/ 50[d]
Fraction 3	1.8	23/ 50	30/ 50
Fraction 4	8.3	44/ 50[d]	55/ 100[d]
Fraction 5	11.8	24/100	27/ 100
Compound I	1.1	51/ 50[d]	78/ 50[d]
Compound II	3.6	53/ 50[d]	34/ 50[d]
Indomethacin		49/ 10[c]	43/ 2.5[c]
Aspirin		27/ 50[c]	90/ 50[d]

a) Inhibitory % at 3 h after injection of carrageenan. —, no effect. *b*) The number of writhings was counted for 5 min beginning from 10 min after *i.p.* injection of acetic acid. —, no effect. *c*) Significant at $p<0.05$. *d*) Significant at $p<0.01$.

Experimental

Melting points were determined on a Yanaco MP-S3 micro melting point apparatus (hot-stage type) and are uncorrected. Optical rotation was measured with a JASCO DIP-140 digital polarimeter. Low-resolution mass spectra (MS) were taken with a JEOL JMS-D 100 instrument. Infrared (IR) spectra were recorded on a JASCO A-220 IR spectrophotometer. Ultraviolet (UV) spectra were recorded on a Shimadzu UV-220 spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were measured with a JEOL FX-90 NMR spectrometer. Chemical shifts are reported in δ-values downfield from internal tetramethylsilane (TMS), and the following abbreviations are used: s = singlet, d = doublet, and q = quartet. The coupling constants are quoted in Hz. Elemental analyses were done with a Perkin Elmer 240C elemental analyzer.

Assay for Anti-inflammatory Activity——A carrageenan-induced inflammatory edema method described by Winter *et al.* was used in these experiments.[4] Details were described in the previous paper.[1] Results were expressed as an inhibition of swelling at 3 h, relative to the control group given the vehicle (1% Tween 80–0.9% NaCl solution).

Assay for Analgesic Activity——An acetic acid-induced writhing method reported by B. A. Whittle[5] was used in these experiments. Details were described in the previous paper.[1] The number of writhings was counted in each animal for 5 min beginning from 10 min after the acetic acid challenge.

Extraction and Partition——The dried whole herb of *Pyrola rotundifolia* L. (1 kg), obtained from the Institute of Chinese Materia Medica, Academy of Chinese Traditional Medicine, China, was extracted three times with 5 l each of methanol under reflux. The mixture was filtered and the filtrate was concentrated *in vacuo*, keeping the temperature below 40 °C, to give the methanol extract (MeOH ext., 104 g). A part of the MeOH ext. (20 g) was dissolved in 1000 ml of water saturated with *n*-butanol, and extracted twice with 1000 ml of *n*-butanol saturated with water. The combined extracts were concentrated *in vacuo*, affording the *n*-butanol-soluble fraction (*n*-BuOH sol., 12.7 g). This distribution was performed repeatedly, and 65.8 g of *n*-BuOH sol. and 38.2 g of water-soluble fraction (H_2O sol.) were obtained from 104 g of the MeOH ext.

Ether Extraction and Chromatography——The *n*-BuOH sol. (65.8 g) was extracted three times with 3 l each of ethyl ether under reflux. The mixture was filtered and the filtrate was concentrated *in vacuo* to give the ether extract (ether ext., 31.4 g). A part of the ether ext. (10.0 g) was subjected to column chromatography on silica gel (500 g, Kieselgel 60, Merck) with *n*-hexane : ethyl acetate = 2 : 1 (v/v) as the eluent, giving five fractions, fr. 1 ($Rf>0.70$, 1.1 g), fr. 2 ($Rf=0.70$—0.50, 0.6 g), fr. 3 ($Rf=0.50$—0.30, 0.6 g), fr. 4 ($Rf=0.30$—0.10, 2.7 g), and fr. 5 ($Rf<0.10$, 3.9 g) based on monitoring by thin layer chromatography (TLC) (solvent system, *n*-hexane : ethyl acetate = 2 : 1, Kieselgel 60 F_{254}, Merck). This chromatography was repeated three times, and 1.8 g of fr. 2 and 8.3 g of fr. 4 were obtained from 31.4 g of the ether ext. Fraction 2 (1.8 g), after recrystallization from *n*-hexane, gave 1.1 g of the active compound I. Fraction 4 (8.3 g), after recrystallization from ethanol, gave 3.6 g of the active compound II.

Compound I (Chimaphilin)——Yellow needles. mp 114.5—115 °C. MS *m/z*: 186 (M^+), 171, 158, 129, 118. IR ν_{max}^{KBr} cm^{-1}: 1665, 1600. UV λ_{max}^{MeOH} nm (log ε): 251 (4.41, sh), 256 (4.44), 262 (4.23, sh), 339 (3.52). ^{1}H-NMR ($CDCl_3$) δ: 2.17 (3H, d, J=1.6), 2.48 (3H, s), 6.78 (1H, q, J=1.6), 7.59 (1H, dd, J=8.0, 1.5), 7.88 (1H, d, J=1.5), 7.92 (1H, d, J=8.0). ^{13}C-NMR ($CDCl_3$) δ: 16.3 (q), 21.9 (q), 126.2 (d), 126.8 (d), 130.1 (s), 132.2 (s), 134.3 (d), 135.7 (d), 144.6 (s), 147.9 (s), 184.7 (s), 185.6 (s). *Anal.* Calcd for $C_{12}H_{10}O_2$: C, 77.40; H, 5.41. Found: C, 77.45; H, 5.43.

Compound II (Ursolic Acid)——Colorless needles. mp 285—287 °C. $[\alpha]_D^{23}$ +71.3° (c=0.35, EtOH). MS *m/z*: 456 (M^+), 438, 411, 248, 207, 203. IR ν_{max}^{KBr} cm^{-1}: 3530, 2950, 1715, 1450, 1120, 1040. ^{13}C-NMR (C_5D_5N) δ: 15.7, 16.5, 17.4, 18.8, 21.3, 23.7, 23.9, 24.9, 28.1, 28.8, 31,1, 33.6, 37.3, 39.2, 39.3, 40.0, 42.5, 48.1, 53.6, 55.9, 78.2, 125.6, 139.2, 179.7. *Anal.* Calcd for $C_{30}H_{48}O_3$: C, 78.90; H, 10.59. Found: C, 78.72; H, 10.58.

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