Platelet Aggregation Inhibitors and Inotropic Constituents in Pyrolae Herba

Kiyomi KAGAWA, Katsuya TOKURA,* Kiyohisa UCHIDA, Hisato KAKUSHI, Tsutomu SHIKE, and Hiroshi NAKAI

Shionogi Research Laboratories, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553, Japan. *Received January 13, 1992*

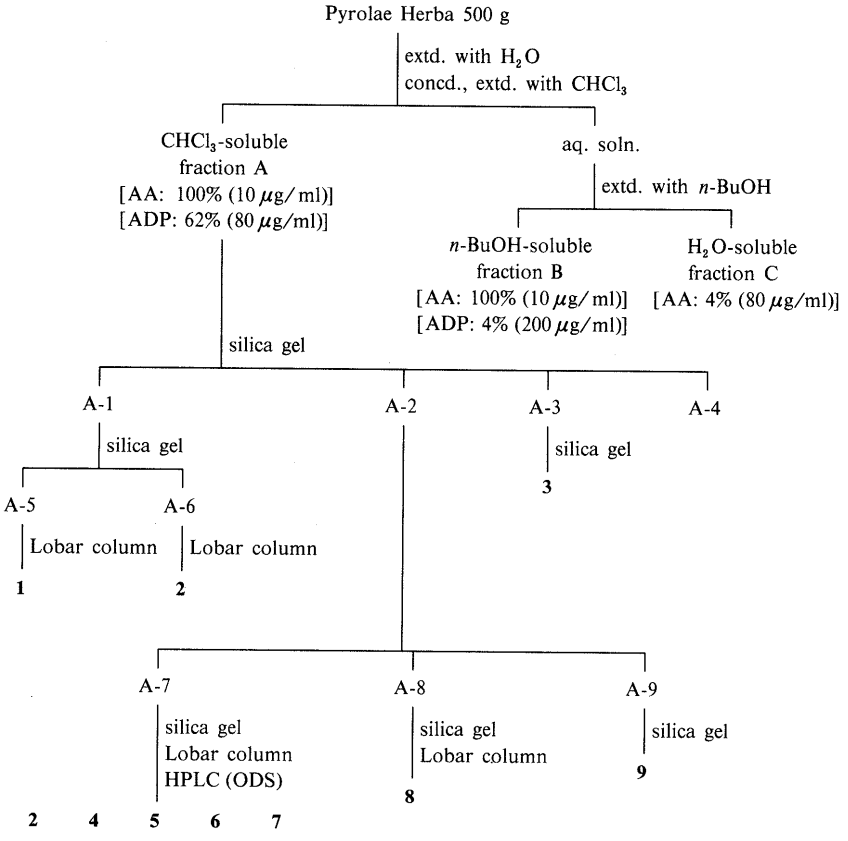
The chloroform-soluble and *n*-butyl alcohol-soluble fractions of water extract of Pyrolae Herba inhibited platelet aggregation induced by arachidonic acid and showed a positive inotropic effect. A new naphthoquinone and a new tetralone derivative and known chimaphilin, acetovanillon, and toluhydroquinone were isolated as active constituents. Three new tetralone derivatives were also obtained from an active fraction. The structures of the new compounds were elucidated.

Keywords Pyrolae Herba; Pyrolaceae; chimaphilin; apocynin; acetovanillon; toluhydroquinone; naphthoquinone; tetralone; platelet aggregation inhibitor; positive inotropic effect

The isolation of pyrocatechol and salicyl alcohol as platelet aggregation inhibitors of *Populus sieboldii* MIQUEL was reported in another paper.[1] Continuing our screening for biologically active constituents from plant sources, we found that chloroform ($CHCl_3$)-soluble and *n*-butyl alcohol (*n*-BuOH)-soluble fractions of the water extract of Pyrolae Herba displayed inhibition of arachidonic acid-induced platelet aggregation and a positive inotropic effect on isolated guinea pig right atria. Here, we describe the isolation of the active constituents and elucidate the structures of new compounds obtained from the active fractions.

Pyrolae Herba (Chinese name: Lu xian cao, Lu han cao, or Lu ti cao), the whole herb of *Pyrola rotundifolia*, *P. incarnata*, *P. japonica*, or other *Pyrola* plants (Pyrolaceae), has been used as tonics, sedatives, analgesics against rheumatoid arthritis, and hemostatics. The crude drug is also used as an anecdote for various poisonous bites of snakes, insects, and dogs.

Isolation of Platelet Aggregation Inhibitors The $CHCl_3$-soluble fraction (A) of the water extract inhibited platelet aggregation induced by arachidonic acid (AA) or adenosine diphosphate (ADP), whereas the *n*-BuOH-soluble fraction (B) inhibited only AA-induced platelet aggregation. More than two platelet aggregation inhibitors having different action mechanisms were thought to be present in this plant. These two fractions were separated further with monitoring of the inhibitory activity, as shown in Fig. 1. Fraction A was separated into four fractions (A-1 to A-4). Fraction A-1 inhibited the platelet aggregation induced by either AA



[ ] indicates inhibition of AA- or ADP-induced platelet aggregation

Fig. 1. Fractionation of Pyrolae Herba Extract

or ADP, and afforded two active compounds **1** and **2**. Compound **1** was identified as chimaphilin (2,7-dimethyl-1,4-naphthoquinone) and **2** as acetovanillon (4-hydroxy-3-methoxy-acetophenone or apocynin) by direct comparison of their physical properties and spectral data with those of authentic samples. Another active constituent (**3**) was obtained from fraction A-3 and was identified as toluhydroquinone (2-methylhydroquinone). Fraction A-2 was less active than fractions A-1 and A-3, but could be separated to give four new tetralone derivatives **4**, **5**, **6**, and **7**. As described below, only **5** showed weak inhibitory activity. The fraction also afforded a new naphthoquinone (**8**) and **9** as active components. Compound **9** was identified as 4-hydroxyacetophenone. The structures of the new compounds are elucidated below.

Fraction B gave **3**, two flavonol glycosides, **10** and **11**, and **12**. Compound **10** was assumed to be hyperin (quercetin-3-*O*-*β*-D-galactoside) from comparison of the proton nuclear magnetic resonance (^{1}H-NMR) spectrum with those of quercetin and quercetin-3-*O*-*β*-D-glucoside. The ^{13}C-NMR spectrum of **10** agreed well with the reported data.[2] ^{1}H-NMR spectrum of **11** coincided with the data reported for quercetin-3-*O*-*β*-D-galactoside-2″-gallate,[3] and its ^{13}C-NMR spectrum was well elucidated from those of **10**, quercetin-3-*O*-*β*-D-galactoside-6″-gallate,[2] and quercetin-3-*O*-*β*-D-glucoside-2″-gallate.[4] Compounds **10** and **11** were isolated from *Pyrola incarnata* and **11** is known to have tanning activity.[3] Compound **12** was identified as homoarbutin (3-methyl-4-hydroxyphenyl-*β*-D-glucoside).

Inhibitory effects of the isolated compounds on AA-induced platelet aggregation are shown compared with that of aspirin (Table I). Inhibition of platelet aggregation induced by ADP, collagen, or U-46619 was also tested with **1** and **2**. Compound **1** inhibited the aggregation induced by ADP (44% at 80 μg/ml), collagen (100% at 20 μg/ml), or U-46619 (75% at 80 μg/ml). Compound **2** inhibited the aggregation induced by collagen (100% at 80 μg/ml), but not the aggregation induced by ADP or U-46619 at the concentration of 80 μg/ml.

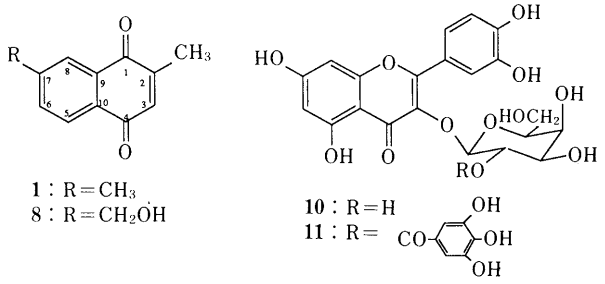


Chart 1

TABLE I. Inhibitory Effect of Pyrolae Herba Constituents on AA-Induced Platelet Aggregation

Compound	Concentration (μg/ml)	Concentration (μM)	Inhibition (%)
1	2	10	100
2	40	240	100
3	1	8	88
4	80		3.8
5	80	420	100
6	80		13.6
7	80		8.2
8	80	400	100
9	20	150	100
10	80		4
11	80		0
12	80		5
Aspirin	18	100	100

Isolation of Positive Inotropic Substances Fractions A and B also exhibited a positive inotropic effect on isolated guinea pig right atria (+63% and +47%, respectively, at the concentration of 10^{-5} g/ml), but fraction C did not. Fractions A and B were separated similarly to the separation of platelet aggregation inhibitors. All the fractions obtained by silica gel chromatography of fraction A exhibited positive inotropic activity. These active fractions afforded compounds **1** to **8**. Fraction B gave compounds **3**, **10**, **11**, and **12**.

The positive inotropic activities of these compounds were tested (Table II). At the concentration of 10^{-5} g/ml, **2**, **4**, **5**, **6**, **7**, **10**, **11**, and **12** did not show inotropic activity, while **1**, **3**, and **8** exhibited strong activity. Commercially available 1,4-naphthoquinone (**13**) and 2-methyl-1,4-naphthoquinone (menadione) (**14**) also showed inotropic activity. Compounds **1**, **3**, and **8** were concerned in both inotropic activity and platelet aggregation inhibitory activity. Compound **1** is also known to be an antiinflammatory and analgesic principle of *Pyrola rotundifolia*.[5]

Structure of a New Naphthoquinone The infrared (IR) spectrum of **8** was quite similar to that of **1**, but indicated the presence of a hydroxyl group (3420 cm^{-1}). ^{1}H-NMR spectrum of **8** exhibited signals at δ 7.74 (dd), 8.06 (d), and 4.85 (s) instead of the signals seen at δ 7.49 (dd), 7.88 (d), and 2.49 (s) in the spectrum of **1**. The singlet at δ 4.85 was shifted to δ 5.24 on acetylation. This shift corresponds to the acetylation shift of a primary alcohol. The signal at δ 64.4 in the ^{13}C-NMR spectrum of **8** supported the presence of a CH_2OH group. Location of the hydroxylmethyl group was confirmed by an X-ray crystallographic structure analysis. The perspective view of the molecule is shown in Fig. 2. Tentative assignment of the ^{13}C-NMR spectrum is shown in Table III compared with those of **1** and **14**.[6]

Structures of New Tetralones Compounds **4**, **5**, **6**, and **7** are thought to be similar to each other judging from their ^{1}H-NMR, IR, and mass (MS) spectra. Compound **4** showed IR absorption at 3580, 3450, and 1683 cm^{-1}. Its ^{1}H-NMR

TABLE II. Inotropic and Chronotropic Effects of Compounds Isolated from Pyrolae Herba

Compound	Concentration (g/ml)	Inotropic maximum response (%)	Chronotropic maximum response (%)
1	10^{-5}	+147.1	+43.9
	10^{-6}	+119.9	+11.7
3	10^{-5}	+131.2	+53.3
	10^{-6}	+2.8	0
8	10^{-5}	+53.5	+1.9
	10^{-6}	+19.2	−7.7
13	10^{-5}	+160.0	+50.8
	10^{-6}	+96.7	+40.9
14	10^{-5}	+172.9	+36.7
	10^{-6}	+19.2	−7.7
Amrinone	10^{-5}	+58.5	+26.9

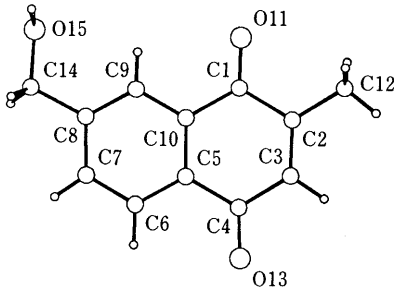


Fig. 2. Perspective View of the Molecule **8**

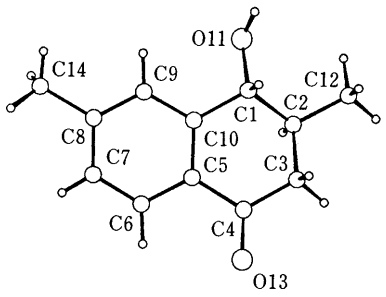


Fig. 3. Perspective View of the Molecule **7**

TABLE III. Tentative Assignments of ^{13}C-NMR Spectra of Naphthoquinones

Carbon	**14**	**1**	**8**
1	184.9	186.5[b]	185.5[e]
2	147.8	145.2	147.1[f]
3	135.4	134.8	135.8
4	184.3	185.6[b]	184.8[e]
5	125.8[a]	126.7[c]	126.6[g]
6	133.3	136.2	131.5
7	133.3	145.2	148.2[f]
8	126.2[a]	127.4[c]	124.3[g]
9	131.9	132.6	131.5[h]
10	131.9	130.5[d]	132.3[h]
2-CH_3	16.3	16.6	16.5
7-CH_3		22.0	
7-CH_2OH			64.4

a—*h*) Assignments may be exchanged.

TABLE IV. Tentative Assignments of ^{13}C-NMR Signals of Compounds **4**—**7**

Carbon	**4**	**5**	Carbon	**6**	**7**
2-CH_3	15.4 q	15.3 q	3-CH_3	16.1 q	18.1 q
7-CH_3	21.0 q	21.2 q	6-CH_3	21.8 q	22.0 q
2	41.3 d	36.8 d	3	34.9 d	38.4 d
3	42.2 t	39.0 t	2	41.2 t	44.0 t
4	68.2 d	66.6 d	4	71.3 d	74.0 d
5	125.9 d	128.4 d	7	127.3 d	127.2 d
8	127.3 d	127.6 d	8	128.8 d	129.0 d
7	130.9 s	131.0 s	9	128.8 s	128.9 s
6	134.6 d	134.7 d	5	129.6 d	127.0 d
9	137.7 s	138.9 s	6	144.0 s[a]	145.2 s[b]
10	143.8 s	140.9 s	10	145.2 s[a]	145.2 s[b]
C=O	199.6 s	200.5 s	C=O	197.6 s	196.9 s

a, *b*) Assignments may be exchanged.

showed signals typical for a 1,2,4-substituted benzene ring at δ 7.61 (d, J=8.0 Hz), 7.42 (dd, J=1.5, 8.0 Hz), and 7.84 (d, J=1.5 Hz). The NMR spectrum also indicated the presence of an aromatic methyl group (δ 2.40, s) and an aliphatic methyl group (δ 1.30, d, J=6.8 Hz). A proton–proton spin decoupling experiment revealed the correlation of signals at δ 2.62 (qdd, J=6.8, 4.7, 11.5 Hz), 2.47 (td, J=4.7, 12.0 Hz), 1.84 (ddd, J=12.0, 11.0, 11.5 Hz), 5.01 (ddd, J=4.7, 7.8, 11.0 Hz), and 2.03 (d, J=7.8 Hz), and suggested the partial structure –CH(OH)–CH_2–CH(CH_3)–. ^{13}C-NMR spectrum of **4** exhibited signals at δ 15.4 (CH_3), 21.0 (CH_3), 41.3 (CH), 42.2 (CH_2), 68.2 (CHOH), and 199.6 (C=O) and signals ascribable to a trisubstituted benzene ring. The nuclear Overhauser effect (NOE) on signals at δ 2.47 and 2.62 was observed on irradiation of the signal at δ 5.01. The presence of strong hydrogen bonding is inconceivable from the carbonyl absorption in the IR spectrum (1683 cm^{-1}) and the NMR signal (δ 2.03) of OH group. Oxidation of **4** with active manganese dioxide (MnO_2) afforded **1**. The relative configuration of **4** was conjectured from these observations and the coupling constants between 2-H_a, 3-H_e, 3-H_a, and 4-H_a.

^{1}H-NMR spectrum of **5** was quite similar to that of **4** and suggested the presence of a 1,2,4-substituted benzene ring. The same partial structure as that for **4** was derived from a proton–proton spin decoupling experiment. Irradiation of the signal at δ 4.97 gave NOE on signals at δ 2.13, 2.34, and 7.33, and irradiation of the signal at δ 7.84 gave NOE on a signal at δ 2.39. The relative configuration was assigned based upon the coupling constants of that partial structure.

The presence of a 1,2,4-substituted benzene ring was

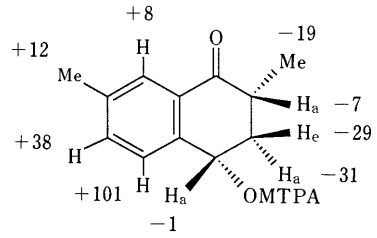
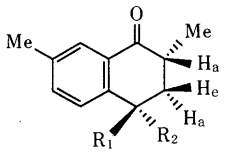
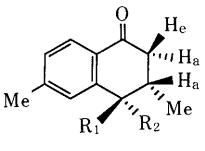


Fig. 4. Δ_δ Values for MTPA Esters of **4**

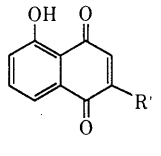
suggested by ^{1}H-NMR spectra of **6** and **7**. The partial structure –CH(OH)–CH(CH_3)–CH_2– was derived from decoupling experiments. NOE on signals at δ 2.44 and 7.28 was observed upon irradiation of the signal at δ 4.78 in **6**. Irradiation of the signal at δ 1.20 gave NOE on signals at δ 2.26, 2.34, 2.85, and 4.54 in **7**. The relative configurations of **6** and **7** were deduced from coupling constants of that partial structure. Assignment of the stereostructure of **7** remained uncertain due to the obscurity of some coupling constants. The structure of **7** was confirmed by X-ray crystallographic analysis, and the perspective view of the molecule is shown in Fig. 3. Tentative assignment of ^{13}C-NMR signals is shown in Table IV. Circular dichroism (CD) spectra of both **4** and **6** showed a positive Cotton effect due to π–π^* transition[7] and suggested the 2*R*, 4*S* configuration for **4** and the 3*S*, 4*S* one for **6**. The absolute stereostructure of **4** was supported by application of an advanced Mosher's method[8] using (*R*)-(+)- and (*S*)-(−)-MTPA [α-methoxy-α-(trifluoromethyl)-phenylacetic acid] esters. $\Delta\delta$ (δ_S–δ_R) values are shown in Fig. 4. The absolute stereochemistry of **7** was elucidated similarly to be 3*S*, 4*R*. However, the assignments were ambiguous in both cases,



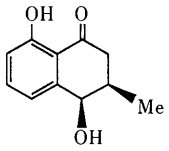
4 : $R_1=H_a$ $R_2=OH$
5 : $R_1=OH$ $R_2=H_e$



6 : $R_1=H_e$ $R_2=OH$
7 : $R_1=OH$ $R_2=H_a$



15 : R=Me
17 : R=H



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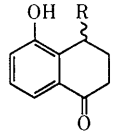

18 : R=β-OH
19 : R=α-OH

Chart 2

because signals due to less favorable conformers were also observed. The absolute stereostructures of **5** remained to be elucidated.

The relation of **1** to these partially reduced forms **4** to **7** is similar to the relation of plumbagin (**15**) to isoshinanolone (**16**)[9] and that of juglone (**17**) to sclerone (**18**) and isosclerone (**19**).[10]

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Ultraviolet (UV) spectra were recorded on a Hitachi EPS-3T spectrophotometer. IR spectra were recorded on a JASCO A-702 infrared spectrometer. The 200 MHz ^{1}H-NMR spectra and 50.3 MHz ^{13}C Fourier transform NMR spectra were observed on a Varian XL-200 or Varian VXR-200 spectrometer. The 400 MHz ^{1}H-NMR spectra were determined on a Varian XL-400 spectrometer. Chemical shifts were expressed as δ (ppm downfield from the internal tetramethylsilane signal). MS were taken with a Hitachi M-68 mass spectrometer. High resolution MS (HR-MS) were taken with a Hitachi M-2000 A mass spectrometer. CD spectra were recorded on a JASCO J-720 spectropolarimeter. Centrifugal partition chromatography (CPC) was carried out with a model CPC-LLN (Sanki Engineering Co.). High performance liquid chromatography (HPLC) was performed with a Knauer type 64 HPLC pump equipped with a UV detector UVIDEC 100-V (Japan Spectroscopic Co.) monitoring the UV absorption at 230 nm. Preparative HPLC was carried out with a Develosil ODS column (Nomura Chemicals Co., 10—20 μm, 20 mm i.d. × 250 mm) using 50% or 70% aq. MeOH as a developing solvent. Silica gel (Silica gel 60, 230—400 mesh, Merck) or prepacked Lobar column (LiChroprep Si 60, size B, Merck) was used for column chromatography.

X-Ray Structure Analysis of 8 Crystal Data: $C_{12}H_{10}O_3$; $M_r=202.2$; monoclinic; $P2_1/c$; $a=3.899(5)$, $b=11.408(11)$, $c=21.672(13)$ Å; $\beta=96.44(7)°$; $V=958(2)$ Å^3; $Z=4$; $D_c=1.402$ g·cm^{-3}; $F(000)=424$.

Yellow prism crystals were grown from *n*-hexane–ethyl acetate. The diffraction intensities were collected from a single crystal with dimensions 0.3 × 0.2 × 0.2 mm on a Rigaku AFC-5R four-circle diffractometer using graphite-monochromated CuK_α radiation. A total of 1639 unique reflections were measured within a 2θ range of 130°, of which 1468 with $F\geq 3\sigma(F_O)$ were considered as observed. The structure was solved by direct method using MULTAN 84[11] and refined by the block-diagonal least squares method. In the final refinement, anisotropic thermal parameters were used for nonhydrogen atoms. The final R value was 0.057. The final atomic coordinates and bond distances are given in Tables V and VI, respectively.

X-Ray Structure Analysis of 7 Crystal Data: $C_{12}H_{14}O_2$; $M_r=190.2$; monoclinic; $P2_1/c$; $a=10.040(2)$, $b=13.119(5)$, $c=8.784(2)$ Å; $\beta=114.08(1)°$; $V=1056.3(5)$ Å^3; $Z=4$; $D_c=1.196$ g·cm^{-3}; $F(000)=408$.

Colorless plate crystals were grown from *n*-hexane–benzene. The diffraction intensities were collected from a single crystal with dimensions 0.4 × 0.5 × 0.03 mm. A total of 1953 unique reflections were measured within a 2θ range of 140°, of which 1542 with $F\geq 3\sigma(F_O)$ were considered as observed. The other features of data collection and structure determination were the same as with **8**. The final R value was 0.049. The final atomic coordinates and bond distances are given in Tables VII and VIII, respectively.

TABLE V. Atomic Coordinates for **8** with Their e.s.d.'s in Parentheses

Atom	x	y	z
C1	0.3038 (3)	−0.2556 (1)	0.8795 (1)
C2	0.4757 (4)	−0.2247 (1)	0.9427 (1)
C3	0.6359 (4)	−0.1211 (1)	0.9518 (1)
C4	0.6566 (3)	−0.0342 (1)	0.9021 (1)
C5	0.4915 (3)	−0.0632 (1)	0.8391 (1)
C6	0.5022 (4)	0.0161 (1)	0.7904 (1)
C7	0.3574 (4)	−0.0133 (1)	0.7317 (1)
C8	0.1947 (3)	−0.1218 (1)	0.7195 (1)
C9	0.1780 (3)	−0.1998 (1)	0.7682 (1)
C10	0.3241 (3)	−0.1712 (1)	0.8279 (1)
O11	0.1486 (4)	−0.3483 (1)	0.8719 (1)
C12	0.4551 (5)	−0.3127 (2)	0.9930 (1)
O13	0.8130 (3)	0.0584 (1)	0.91343 (5)
C14	0.0469 (4)	−0.1499 (1)	0.6538 (1)
O15	−0.1440 (3)	−0.2546 (1)	0.6478 (1)

TABLE VI. Bond Distances (Å) of **8** with Their e.s.d.'s in Parentheses

Bond	Distance	Bond	Distance
C1–C2	1.498 (3)	C5–C6	1.394 (3)
C1–C10	1.485 (3)	C5–C10	1.403 (3)
C1–O11	1.221 (3)	C6–C7	1.374 (3)
C2–C3	1.341 (3)	C7–C8	1.403 (3)
C2–C12	1.491 (3)	C8–C9	1.388 (3)
C3–C4	1.473 (3)	C8–C14	1.509 (3)
C4–C5	1.480 (3)	C9–C10	1.393 (3)
C4–O13	1.231 (2)	C14–O15	1.406 (3)

TABLE VII. Atomic Coordinates for **7** with Their e.s.d.'s in Parentheses

Atom	x	y	z
C1	1.0326 (2)	0.2793 (1)	1.1868 (2)
C2	1.1309 (2)	0.1862 (1)	1.2114 (2)
C3	1.0497 (2)	0.0921 (1)	1.2301 (2)
C4	0.9034 (2)	0.0768 (1)	1.0913 (2)
C5	0.8283 (2)	0.1676 (1)	0.9938 (2)
C6	0.6954 (2)	0.1559 (1)	0.8569 (2)
C7	0.6211 (2)	0.2402 (2)	0.7705 (2)
C8	0.6752 (2)	0.3373 (1)	0.8171 (2)
C9	0.8082 (2)	0.3483 (1)	0.9500 (2)
C10	0.8873 (2)	0.2648 (1)	1.0402 (2)
O11	1.1017 (1)	0.3686 (1)	1.1621 (2)
C12	1.2759 (2)	0.1982 (2)	1.3612 (3)
O13	0.8447 (2)	−0.0072 (1)	1.0618 (2)
C14	0.5894 (3)	0.4289 (2)	0.7233 (4)

Platelet Aggregation Platelet aggregation was examined by the method of Born[12] using a Type 61 Autoram, Aggregometer (Rika-Denki Co., Ltd. Tokyo).[13]

Inotropic Activity Inotropic and chronotropic effects were isotonically determined using isolated guinea pig right atria according to the Magnus method.

Extraction and Isolation Pyrolae Herba (500 g) purchased from Tochimoto-tenkaido, Osaka, was extracted with hot water three times. The combined extract was concentrated and partitioned with $CHCl_3$ and *n*-BuOH successively. Fraction A ($CHCl_3$) was separated by silica gel chromatography into four fractions, A-1 (89 mg), A-2 (79 mg), A-3 (65 mg), and A-4 (24 mg). Repeated chromatography of fraction A-1 afforded **1** (15 mg) and **2** (20 mg). Compound **3** (11 mg) was obtained from fraction A-3 by silica gel chromatography. An additional amount of fraction A-2 (1.45 g) was obtained together with 241 mg of **1** and 218 mg of **2** starting

TABLE VIII. Bond Distances (Å) of **7** with Their e.s.d.'s in Parentheses

Bond	Distance	Bond	Distance
C1–C2	1.529 (3)	C5–C6	1.393 (3)
C1–C10	1.513 (3)	C6–C10	1.395 (3)
C1–O11	1.423 (3)	C6–C7	1.376 (3)
C2–C3	1.525 (3)	C7–C8	1.380 (3)
C2–C12	1.521 (3)	C8–C9	1.376 (3)
C3–C4	1.491 (3)	C8–C14	1.512 (4)
C4–C5	1.481 (3)	C9–C10	1.395 (3)
C4–O13	1.226 (3)		

from 2.7 kg of the crude drug. Fraction A-2 was separated into three active fractions (A-7 to A-9). Separation of fraction A-7 by silica gel chromatography and reversed phase HPLC gave **2** (78 mg), **4** (33 mg), **5** (34 mg), **6** (49 mg), and **7** (18 mg). On repeated chromatography on silica gel, fraction A-8 afforded **8** (84 mg) and fraction A-9 gave **9** (24 mg). Fraction B was subjected to CPC (AcOEt–EtOH–H_2O, 5:1:5) to give fractions B-1 (935 mg), B-2 (133 mg), B-3 (606 mg), and B-4 (130 mg). Crystallization of AcOEt-soluble part of fraction B-3 gave **12** (147 mg). Preparative reversed phase HPLC of fraction B-1 gave **3** (127 mg), **10** (46 mg), and **11** (106 mg).

Chimaphilin (1) Yellow needles from *n*-hexane, mp 113—114 °C. This compound was identified by comparison with an authentic sample supplied by Prof. H. Inouye.

Acetovanillon (2) Colorless prisms from *n*-hexane–AcOEt or H_2O, mp 113—115 °C. mp and IR and NMR spectra were identical to those of a commercially available sample.

Toluhydroquinone (3) Colorless plates, mp 125—128 °C. This compound was identical with an authentic sample supplied by Prof. Inouye.

Compound 4 Colorless needles from *n*-hexane–benzene, mp 109—111 °C. MS *m/z*: 190 (M^+), 161, 148, 119, 91. UV λ_{max}^{MeOH} (log ε): 250 (4.03), 299 (3.28). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 3450 (br), 1683, 1612. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, d, J=6.8 Hz), 1.84 (1H, ddd, J=12.0, 11.5, 11.0 Hz), 2.03 (1H, d, J=7.8 Hz), 2.40 (3H, s), 2.47 (1H, td, J=4.7, 12.0 Hz), 2.62 (1H, qdd, J=6.8, 4.7, 11.5 Hz), 5.01 (1H, ddd, J=11.0, 7.8, 4.7 Hz), 7.42 (1H, dd, J=8.0, 1.5 Hz), 7.61 (1H, d, J=8.0 Hz), 7.84 (1H, d, J=1.5 Hz). CD (c=0.015, MeOH) [θ] (nm): 0 (380), +4160 (321.5) (positive maximum), 0 (309), −6810 (292) (negative maximum), −1060 (260 sh), 0 (245). *Anal.* Calcd for $C_{12}H_{14}O_2$: C, 75.76; H, 7.42. Found: C, 75.41; H, 7.48.

MnO_2 Oxidation of 4 Compound **4** (10 mg) and active MnO_2 (80 mg) were refluxed in benzene (6 ml) for 5 h. The reaction mixture was filtered and the filtrate was evaporated *in vacuo*. Chromatographic separation (silica gel, 2.5 g) and crystallization from *n*-hexane afforded yellow needles (7 mg), which were identified with those of **1**.

Compound 5 Colorless oil. MS *m/z*: 190 (M^+), 161, 148, 119, 91. HR-MS *m/z* Calcd for $C_{12}H_{14}O_2$ (M^+): 190.0994. Found: 190.0993. UV λ_{max}^{MeOH} (log ε): 250 (3.97), 296 (3.17). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 3420 (br), 1683, 1610. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (1H, d, J=6.8 Hz), 1.89 (1H, br s), 2.13 (1H, ddd, J=13.5, 11.2, 3.6 Hz), 2.34 (1H, td, J=4.7, 13.5 Hz), 2.39 (3H, s), 3.12 (1H, qdd, J=6.8, 11.2, 4.7 Hz), 4.97 (1H, dd, J=4.7, 3.6 Hz), 7.33 (1H, d, J=7.5 Hz), 7.40 (1H, dd, J=7.5, 1.5 Hz), 7.84 (1H, d, J=1.5 Hz). CD (c=0.01, MeOH) [θ] (nm): 0 (370), +80 (320), 0 (308), −320 (292), −90 (270).

Compound 6 Colorless oil. MS *m/z*: 190 (M^+), 148, 119, 91. HR-MS *m/z* Calcd for $C_{12}H_{14}O_2$ (M^+): 190.0994. Found: 190.0992. UV λ_{max}^{MeOH} (log ε): 258 (4.15). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 3420 (br), 1680, 1610. ^{1}H-NMR ($CDCl_3$) δ: 1.15 (1H, d, J=6.7 Hz), 1.89 (1H, br d, J=3.8 Hz), 2.42 (3H, s), 2.44 (1H, m), 2.53 (1H, dd, J=17.0, 3.5 Hz), 2.78 (1H, dd, J=17.0, 11.0 Hz), 7.23 (1H, dd, J=8.0, 1.5 Hz), 7.28 (1H, d, J=1.5 Hz), 7.92 (1H, d, J=8.0 Hz). CD (c=0.014, MeOH) [θ] (nm): 0 (380), +820 (345 sh), +1200 (333) (positive maximum), +1070 (323) (positive maximum), +700 (313.5), +2310 (293.5) (positive maximum), 0 (260), +750 (242), 0 (230).

Compound 7 Colorless needles from *n*-hexane–benzene, mp 75—76 °C. MS *m/z*: 190 (M^+), 161, 148, 119, 91. UV λ_{max}^{MeOH} (log ε): 258 (4.16). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 3420 (br), 1680, 1610. ^{1}H-NMR ($CDCl_3$) δ: 1.20 (3H, d, J=6.7 Hz), 2.06 (1H, d, J=7.0 Hz), 2.26 (1H, m), 2.34 (1H, m), 2.43 (3H, s), 2.85 (1H, dd, J=16.0, 3.5 Hz), 4.54 (1H, dd, J=7.8, 7.0 Hz), 7.21 (1H, dd, J=7.8, 1.5 Hz), 7.49 (1H, d, J=1.5 Hz), 7.90 (1H, d, J=7.8 Hz). CD (c=0.013, MeOH) [θ] (nm): 0 (360), −160 (300), −210 (280). *Anal.* Calcd for $C_{12}H_{14}O_2$: C, 75.76; H, 7.42. Found: C, 75.68; H, 7.47.

2-Methyl-7-hydroxymethyl-1,4-naphthoquinone (8) Yellow needles from benzene, mp 128.5—129.5 °C. MS *m/z*: 202 (M^+), 174, 173, 145, 134, 105. UV λ_{max}^{MeOH} (log ε): 254 (4.36), 336 (3.54). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3601, 3412 (br), 1675, 1661, 1622, 1603. ^{1}H-NMR ($CDCl_3$) δ: 2.19 (3H, d, J=1.6 Hz), 4.86 (2H, s), 6.82 (1H, q, J=1.6 Hz), 7.74 (1H, dd, J=8.0, 1.5 Hz), 8.05 (1H, d, J=8.0 Hz), 8.08 (1H, d, J=1.5 Hz). ^{13}C-NMR ($CDCl_3$): see Table III. *Anal.* Calcd for $C_{12}H_{10}O_3$: C, 71.28; H, 4.99. Found: C, 71.25; H, 5.00.

Acetylation of 8 Compound **8** (5 mg) was acetylated with pyridine (0.2 ml) and acetic anhydride (0.2 ml) at room temperature. Chromatography on silica gel and crystallization from *n*-hexane–benzene afforded 6 mg of yellow needles, mp 127—129 °C. IR $\nu_{max}^{CHCl_3}$: 1739, 1663, 1605. ^{1}H-NMR ($CDCl_3$) δ: 2.18 (3H, s), 2.23 (3H, d, J=1.6 Hz), 5.24 (2H, s), 6.87 (1H, q, J=1.6 Hz), 7.71 (1H, dd, J=8.0, 1.8 Hz), 8.08 (1H, d, J=8.0 Hz), 8.09 (1H, d, J=1.8 Hz).

4-Hydroxyacetophenone (9) Colorless needles from benzene, mp 109—110 °C. mp and IR and NMR spectra agreed with those of a commercially available sample.

Hyperin (10) Yellow needles from MeOH, mp 224—247 °C. This compound was identified with an authentic sample by Prof. T. Okuda.

Compound 11 Yellow needles from H_2O, mp 199—201 °C. This compound was identified with an authentic sample by Prof. T. Okuda.

Homoarbutin (12) Colorless needles from $CHCl_3$–MeOH, mp 185—189 °C. This compound was identical with an authentic sample supplied by Prof. H. Inouye.

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