Indonesian Medicinal Plants. II.[1] Chemical Structures of Pongapinones A and B, Two New Phenylpropanoids from the Bark of *Pongamia pinnata* (Papilionaceae)

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Two new phenylpropanoids named pongapinone A (1) and pongapinone B (2) were isolated from the bark of *Pongamia pinnata* (Papilionaceae), an Indonesian medicinal plant, and their chemical structures have been elucidated on the basis of their physicochemical properties. Pongapinone A (1) was found to inhibit interleukin-1 production.

Keywords Indonesian medicinal plant; *Pongamia pinnata*; Papilionaceae; phenylpropanoid; pongapinone; β-hydroxychalcone; flavanone

Pongamia pinnata (Papilionaceae) is called "Warrt" in the Larantuka area of Flores Island, Indonesia. The decoction of the bark is drunk after childbirth and the pounded bark mixed with the seeds of *Aleurites moluccana* (Euphorbiaceae) and *Areca catechu* (Arecaceae) has been used to cure swelling caused by stinging fish.[2] As for the chemical constituents in this plant, several phenylpropanoids such as flavones,[3] flavanones,[4] chalcones,[5] and β-hydroxychalcones[4a,6] have so far been identified from the plant collected in various countries. During our scientific expedition in search of Indonesian medicinal plants in 1988, we collected the plant *Pongamia pinnata* in the Larantuka area. Through bioactivity-directed fractionation and separation of the extract, we have found a new pyrano-β-hydroxychalcone designated pongapinone A (**1**), which inhibits the production of interleukin-1, and a new prenylflavanone named pongapinone B (**2**) from the bark. This paper deals with the structure elucidation of these phenylpropanoids.

The methanol extract of the bark was partitioned into an ethyl acetate and water solvent system to separate an ethyl acetate-soluble portion (1.5% yield from the bark). In preliminary examinations, the ethyl acetate-soluble portion was found to contain constituents having activity to inhibit the production of interleukin-1.[7] The ethyl acetate-soluble portion was then subjected to silica gel column chromatography with monitoring of the bioactivity to afford pongapinone A (**1**, 0.038%) as the major active constituent of this portion. The water phase separated from the ethyl acetate phase was further partitioned into *n*-butanol and water to provide an *n*-butanol-soluble portion (1.6% yield) and a water-soluble portion (3.0% yield). The *n*-butanol-soluble portion was further treated with $CHCl_3$ to provide a $CHCl_3$-soluble portion (0.47%). From the $CHCl_3$-soluble portion, pongapinone B (**2**, 0.010%) was isolated by successive silica gel and Sephadex column chromatography.

Pongapinone A (**1**), a yellowish oil, colored orange with $FeCl_3$ reagent on a thin-layer chromatogram (TLC) and gave a molecular ion peak at *m/z* 410 ($C_{23}H_{22}O_7$) in the high resolution electron impact mass spectrum (EI-MS). The infrared (IR) spectrum of **1** showed a characteristic absorption band (1603 cm^{-1}) ascribable to a carbonyl group which was strongly chelated with a hydroxyl group. Furthermore, the ultraviolet (UV) spectrum of **1** showed three maxima at 230, 282 and 375 nm, attributed to a chalcone skeleton.[8] From these spectroscopic properties, it has been presumed that pongapinone A (**1**) possesses a β-hydroxychalcone skeleton. This presumption was supported by the fragment ion peaks observed at *m/z* 247 (**a**) and 149 (**b**) in the EI-MS.[9]

The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of pongapinone A (**1**) exhibited the presence of one 1,3,4-trisubstituted aryl group [δ 6.85 (1H, d, J=8.2 Hz), 7.40 (1H, d, J=1.6 Hz), 7.52 (1H, dd, J=1.6, 8.2 Hz)], one α,α-dimethyldihydropyran moiety [δ 1.45 (6H, s), 5.54 (1H, d, J=9.9 Hz), 6.53 (1H, d, J=9.9 Hz)], two methoxyl groups [δ 3.78, 3.79 (3H each, both s)], one

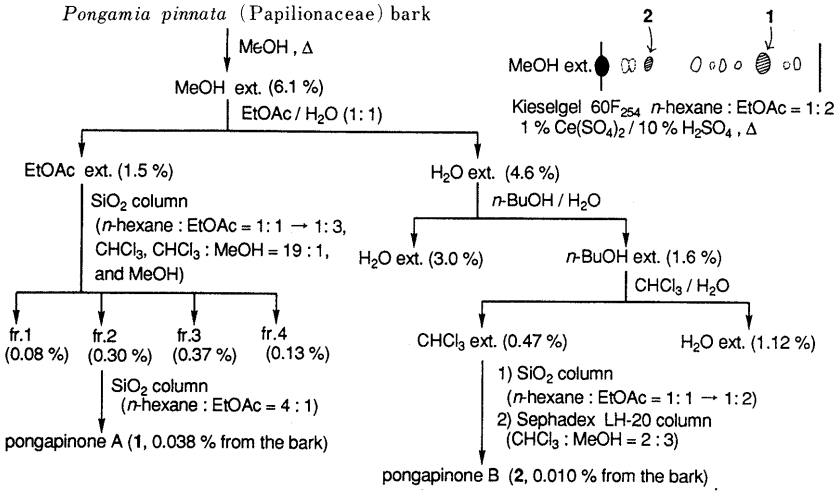
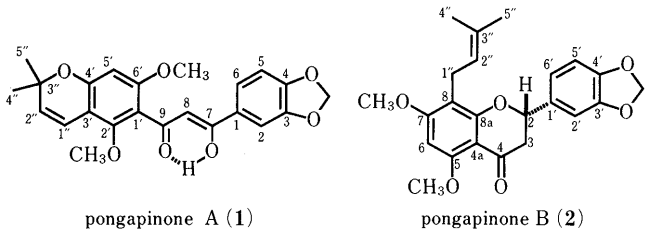
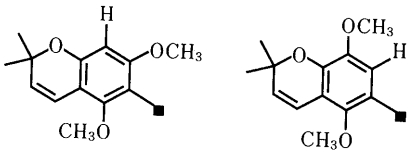


Chart 1



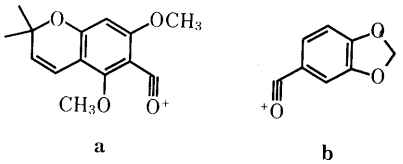
pongapinone A (**1**) pongapinone B (**2**)

Fig. 1



1a **1b**

Fig. 2



a **b**

Fig. 3

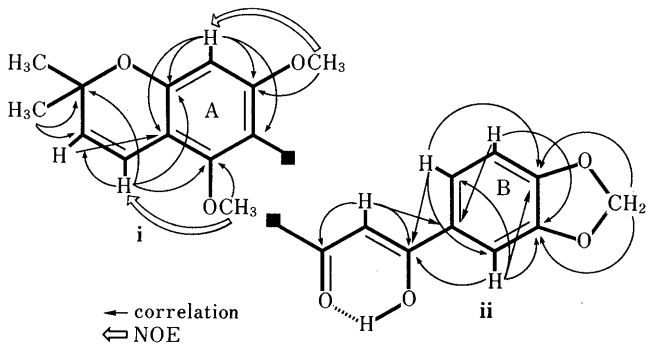


Fig. 4

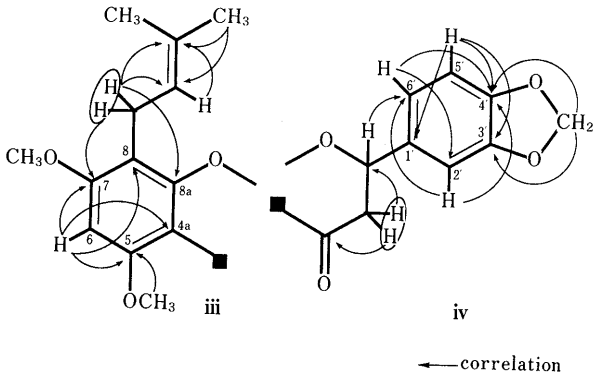


Fig. 5

methylenedioxy group [δ 6.04 (2H, s)], and one β-hydroxy-α,β-unsaturated ketone moiety [δ 6.37 (1H, s), 16.41 (1H, br s)]. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** showed two signals in the carbonyl-carbon range, at δ_C 182.9 (s) and 185.4 (s), which were assignable respectively to C-7 and C-9 in the β-hydroxychalcone skeleton.[10] The former carbon signal was correlated with the signals of H-2 (δ 7.40) and H-6 (δ 7.52) in the correlation spectroscopy *via* long-range coupling (COLOC) spectrum. The chemical shifts (δ_C 155.1 for 2′-C, 156.2 for 4′-C, 158.2 for 6′-C) of aromatic carbons bearing two methoxyl groups and the oxygen in the dimethyldihydropyran ring, suggested that the A-ring of pongapinone A (**1**) is oxygenated in a phloroglucinol manner.[10] These findings have led us to presume that the partial structure of pongapinone A can be depicted as **1a**, or less probably, as **1b**.

In order to determine the structure of pongapinone A (**1**), we next examined the COLOC and the differential nuclear Overhauser effect (NOE) spectra. The COLOC spectrum of **1** showed that the aromatic proton (5′-H) in the A-ring correlated with the β-carbon (1′-C) linked with the carbonyl group (9-C) and the other β-carbon (3′-C), which is in the dimethyldihydropyran ring, but not with the α-carbon (6′-C) having a methoxyl group and the other α-carbon (4′-C), which is also in the dimethyldihydropyran ring. The other correlations observed are shown in **i**. Furthermore, NOEs in the ^{1}H-NMR spectrum of **1** were observed between the signals of one aromatic proton (5′-H) and one methoxyl proton (6′-OCH_3) and between those of the other methoxyl proton (2′-OCH_3) and one olefinic proton (1″-H) in the dimethyldihydropyran ring. Thus, the partial structure (including the A-ring) of pongapinone A (1) has been elucidated as **1a**. On the other hand, the COLOC spectrum of **1** supported the partial structure **ii** (including the B-ring) for the rest of **1**. Consequently, the structure of pongapinone A (**1**) has been determined to be as shown.

Pongapinone B (**2**), obtained as colorless needles, showed typical absorptions in the IR and UV spectra which were reminiscent of a flavanone skeleton of **2**. The ^{1}H-NMR spectrum of **2** showed the presence of one 1,3,4-trisubstituted aryl group [δ 6.96 (1H, d, J=1.6 Hz), 6.88 (1H, dd, J=1.6, 8.2 Hz), 6.81 (1H, d, J=8.2 Hz)], one methylenedioxy group [δ 5.98 (2H, s)], and two methoxyl groups [δ 3.90, 3.93 (3H each, both s)], which were similar to the functional groups elucidated in the A- and B-rings of pongapinone A (**1**). In addition, the ^{1}H-NMR spectrum of **2** showed signals attributable to an isoprenyl group [δ 1.64, 1.66 (3H each, both s), 3.26 (2H, d, J=6.9 Hz), 5.14 (1H, t-like)] and to the central dihydropyrone-ring of a flavanone skeleton [δ 2.79 (1H, dd, J=3.3, 16.5 Hz, 3β-H), 2.94 (1H, dd, J=12.5, 16.5 Hz, 3α-H), 5.30 (1H, dd, J=3.3, 12.5 Hz, 2-H)].

The COLOC spectrum of pongapinone B (**2**) revealed that the allylic methylene protons (δ 3.26, 1″-H_2) were correlated with 7-C (δ_C 163.3) and 8a-C (δ_C 161.0). So, it has been clarified that the isoprenyl moiety attached to the A-ring of **2** is joined to 8-C. The other correlations observed in the COLOC experiment are summarized in **iii** and **iv**. Furthermore, the circular dichroism (CD) spectrum of **2** showed a negative maximum at 292 nm (θ = −22700), indicating the *S*-configuration[11] at the C-2 position.

Based on the foregoing evidence, the chemical structure of pongapinone B has been determined to be **2**.

In conclusion, we have isolated two new phenylpropanoids, pongapinone A (**1**) and pongapinone B (**2**) from the bark of *Pongamia pinnata* collected in the Flores Island of

Indonesia. It was found that pongapinone A (**1**) exhibits an inhibitory activity upon interleukin-1 production (IC_{50} 2.5 μg/ml), [7] which may be related to the medicinal use of the bark.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as in our previous paper.[1]

Plant Materials *Pongamia pinnata* (Papilionaceae) was collected in the Larantuka area of Flores Island, Province Nusa Tenggara Timur, Indonesia, in August 1988. The plant was identified at Herbarium Bogoriense, Research and Development Centre for Biology-LIPI, Indonesia. Voucher specimens have been deposited at Herbarium Bogoriense and the Faculty of Pharmaceutical Sciences, Osaka University.

Isolation of Pongapinones A (1) and B (2) The dried bark (4 kg) of *Pongamia pinnata* was extracted three times with methanol (8 l each) under reflux. The solvent was evaporated off under reduced pressure to yield the MeOH extract (244 g, 6.1% from the bark). The MeOH extract was partitioned into an ethyl acetate–water (1 : 1) mixture. The ethyl acetate phase was separated and concentrated under reduced pressure to give the EtOAc extract (59 g, 1.5%), while the water phase was treated with *n*-butanol. The solvent from both phases was evaporated off under reduced pressure to give the *n*-butanol extract (62 g, 1.6%) and the H_2O extract (120 g, 3.0%). The *n*-butanol extract (21 g) was further partitioned into a mixture of $CHCl_3$ and water. The $CHCl_3$ phase was taken and concentrated under reduced pressure to give the $CHCl_3$ extract (6.2 g, 0.47%).

The EtOAc extract (35 g) was subjected to column chromatography (SiO_2 2 kg, eluted with *n*-hexane : EtOAc = 1 : 1 → 1 : 3, $CHCl_3$, $CHCl_3$: MeOH = 19 : 1, and MeOH) to afford fr. 1 (3.2 g), fr. 2 (11.9 g), fr. 3 (14.7 g), fr. 4 (5.1 g). Purification of fr. 2 (1.19 g) by column chromatography (SiO_2 100 g, *n*-hexane : EtOAc = 4 : 1) afforded pongapinone A (**1**, 98 mg, 0.038% from the bark).

On the other hand, the $CHCl_3$ extract (3.2 g) was purified by successive column chromatography [1) SiO_2 1 kg, *n*-hexane : EtOAc = 1 : 1 → 1 : 2, and 2) Sephadex LH-20, $CHCl_3$: MeOH = 2 : 3] to afford pongapinone B (**2**, 76 mg, 0.010% from the bark).

Pongapinone A (**1**): A yellowish oil. IR ($CHCl_3$) cm^{-1}: 1603, 1572, 1362, 1254. UV (MeOH) nm (ε): 230 (21500), 282 (9500), 375 (20500). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.45 (6H, s, 3″-CH_3 × 2), 3.78 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 5.54 (1H, d, J = 9.9 Hz, 2″-H), 6.04 (2H, s, –OCH_2O–), 6.24 (1H, s, 5′-H), 6.37 (1H, s, 8-H), 6.53 (1H, d, J = 9.9 Hz, 1″-H), 6.85 (1H, d, J = 8.2 Hz, 5-H), 7.40 (1H, d, J = 1.6 Hz, 2-H), 7.52 (1H, dd, J = 1.6, 8.2 Hz, 6-H), 16.41 (1H, br s, 7-OH). ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ_C: 27.8 (totally 2C, q, 4″-C and 5″-C), 55.9 (q, OCH_3), 63.0 (q, OCH_3), 76.8 (s, 3″-C), 96.1 (d, 5′-C), 99.8 (d, 8-C), 101.7 (t, –OCH_2O–), 107.0 (d, 2-C), 108.0 (s, 1′-C), 108.0 (d, 5-C), 113.8 (s, 3′-C), 116.4 (d, 1″-C), 122.7 (d, 6-C), 127.6 (d, 2″-C), 129.6 (s, 1-C), 148.0 (s, 3-C), 151.1 (s, 4-C), 155.1 (s, 2′-C), 156.2 (s, 4′-C), 158.2 (s, 6′-C), 182.9 (7-C), 185.4 (9-C). EI-MS m/z (%): 410 (M^+, 27), 395 ($M^+ - CH_3$, 100), 379 ($M^+ - OCH_3$, 84), 247 (**a**, 27), 149 (**b**, 67). High-resolution EI-MS m/z: Calcd for $C_{23}H_{22}O_7$: 410.1359. Found: 410.1361 (M^+).

Pongapinone B (**2**): Colorless needles, mp 126—127 °C (*n*-hexane–EtOAc). $[\alpha]_D^{21}$ −13.9° (c = 1.02, $CHCl_3$). IR (KBr) cm^{-1}: 1672, 1594, 1258. UV (MeOH) nm (ε): 235 (31700), 288 (30900), 320 (sh). CD ($c = 1.33 \times 10^{-3}$, MeOH): $[\theta]_{292}^{20}$ −22700 (negative max.), $[\theta]_{332}^{20}$ +8700 (positive max.). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.64, 1.66 (3H each, both s, 3″-CH_3 × 2), 2.79 (1H, dd, J = 3.3, 16.5 Hz, 3β-H), 2.94 (1H, dd, J = 12.5, 16.5 Hz, 3α-H), 3.26 (2H, d, J = 6.9 Hz, 1″-H), 3.90 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 5.14 (1H, t-like, 2″-H), 5.30 (1H, dd, J = 3.3, 12.5 Hz, 2-H), 5.98 (2H, s, –OCH_2O–), 6.12 (1H, s, 6-H), 6.81 (1H, d, J = 8.2 Hz, 5′-H), 6.88 (1H, dd, J = 1.6, 8.2 Hz, 6′-H), 6.96 (1H, d, J = 1.6 Hz, 2′-H). ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ_C: 17.7 (q, 4″-C), 21.9 (t, 1″-C), 25.8 (q, 5″-C), 45.7 (t, 3-C), 55.7 (q, OCH_3), 56.0 (q, OCH_3), 78.4 (d, 2-C), 88.6 (d, 6-C), 101.2 (t, –OCH_2O–), 105.9 (s, 8-C), 106.7 (d, 2′-C), 108.2 (d, 5′-C), 110.2 (s, 4a-C), 119.6 (d, 6′-C), 122.5 (d, 2″-C), 131.4 (s, 3″-C), 133.2 (s, 1′-C), 147.6 (s, 4′-C), 147.9 (s, 3′-C), 160.7 (s, 5-C), 161.0 (s, 8a-C), 163.3 (s, 7-C), 190.0 (s, 4-C). EI-MS m/z (%): 396 (M^+, 69), 381 (11), 353 (38), 83 (100). High-resolution EI-MS m/z: Calcd for $C_{23}H_{24}O_6$: 396.1566. Found: 396.1559 (M^+).

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