Two New Acidic Diterpenoids from the Heartwood of *Pinus massoniana* LAMB.

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A new abietane diterpene, named abietopinoic acid, and a new podocarpane diterpene, named podopinoic acid, were isolated from the acetone extract of the heartwood of *Pinus massoniana*. Their structures were established as 12-hydroxy-7-oxoabieta-8,11,13-trien-18-oic acid (**1**) and 13-hydroxy-7-oxopodocarpane (**2**) by means of spectroscopic analyses including 2D-NMR. To the best of our knowledge, this is the second report of a podocarpa-8,11,13-trien-18-oic acid diterpene, isolated from the genus *Pinus*.

Introduction. – The genus *Pinus* (Pinaceae) comprises 250 species and is widespread in the northern hemisphere, especially in the Mediterranean region, Caribbean area, Asia, Europe, and North and Central America [1]. The medicinal and aromatic properties of the chemical compounds (*e.g.*, turpentine, resins, and essential oils) of pine render it one of the most popular plants throughout all cultures. Pine is also still widely used in traditional therapeutic practice throughout the world and is of economic importance [2]. Many coniferous species of the pine family (Pinaceae) contain copious amounts of oleoresin, composed mainly of monoterpenes and diterpene resin acids, in addition to sesquiterpenes [3]. The chemical composition of various pine species has been the subject of numerous studies, the majority of which was focused on North American and Central European species and only a limited number of chemically oriented reports dealt with Mediterranean pine species [4]. The phytochemical study of several species of genus *Pinus* led to the isolation of a number of biologically active compounds. The more common secondary metabolites in this genus have been shown

to be flavonoids, procyanidins, phenols, aromatics, lignans [5–14], monoterpenes [15], diterpenoids [16], and triterpenes [17][18]. Several constituents and extracts of *Pinus* species showed antioxidant, antimicrobial, and antibacterial activities [19][20].

There are four *Pinus* species indigenous to Taiwan, *i.e.*, *P. massoniana*, *P. armandii* var. *masteriana*, *P. morrisonicola*, and *P. taiwanensis*. *P. massoniana* is distributed in the northern and eastern mountain area of Taiwan below 1,500 m. The resin exuded from the trunk of *P. massoniana* has been a traditional medication. The acidic fraction of the resin has been proven to exhibit antithromobotic and antiplatelet-aggregation activities by *Liu et al*. [21]. Earlier studies of *P. massoniana* resulted in the isolation of several acidic diterpenes [22]. Podocarpane diterpenes do not occur abundantly in nature but are present in several genera, such as *Azadirachta* [23–27], *Humirianther* [28], *Micrandropsis* [29], and *Podocarpus* [30]. Herein, we report on a new abietane diterpene, 12-hydroxy-7-oxoabieta-8,11,13-trien-18-oic acid (**1**), and a new podocarpane diterpene, 13-hydroxy-7-oxopodocarpa-8,11,13-trien-18-oic acid (**2**; *Fig. 1*), isolated from the acetone extract of the heartwood of *P. massoniana*.

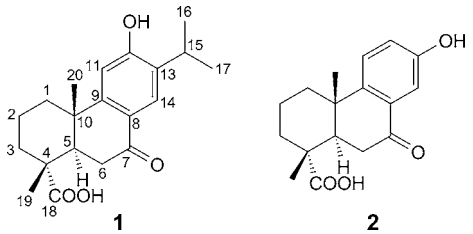

Fig. 1. *Structures of compounds* **1** *and* **2** *isolated from* Pinus massoniana

Results and Discussion. – The UV spectrum of **1** showed absorption bands at λ_{max} 232, 281, and 302 (sh) nm, attributed to a 4-hydroxybenzoyl moiety. Its IR spectrum exhibited absorption bands for COOH, conjugated C=O, and aryl groups at 3334, 3330–2500, 1710, 1673, 1605, and 1510 cm^{-1}. The ^{1}H-NMR spectrum of **1** (*Table*) showed resonances of two aromatic H-atoms in *para*-relation (δ(H) 6.75 (*s*, H–C(11)) and 7.77 (*s*, H–C(14))), an ^{i}Pr group (δ(H) 1.14 (*d*, J = 7.0, Me), 1.15 (*d*, J = 7.0, Me), and 3.17 (*sept.*, J = 7.0, CH)), and two quaternary Me groups (δ(H) 1.18, 1.25 (2*s*, Me(20) and Me(19))). These assignments were confirmed by its ^{13}C- and further ^{1}H-NMR data ((δ(C) 196.0 (*s*), 180.0 (*s*), 108.6 (*d*), 158.9 (*s*), 145.7 (*s*), 132.4 (*s*), 125.3 (*d*), 122.8 (*s*) and δ(H) 6.75 and 7.77 (*Table*))), and the ^{1}H,^{1}H-COSY correlations between the signals of H–C(15) (δ(H) 3.17) and the Me H-atoms (δ(H) 1.18, 1.25). The ^{13}C-NMR spectrum displayed 20 C-atom signals, which assigned by DEPT experiments to four Me, four aliphatic CH_2, and two aliphatic CH groups, two aliphatic quaternary C-atoms, two olefinic CH groups, and four olefinic quaternary and two CO C-atoms. The HR-EI-MS of **1** displayed the molecular-ion peak at *m*/*z* 330.1837, which corresponded to the molecular formula $C_{20}H_{26}O_4$, indicating eight degrees of unsaturation. Since six of eight degrees of unsaturation in compound **1** can be accounted for by the established presence of an aryl ring and two C=O groups, the

Table. *^{1}H- and ^{13}C-NMR Data*[a]) *for* **1** *and* **2**

Position	**1**[b])		**2**[c])	
	δ(H)	δ(C)	δ(H)	δ(C)
1	1.31–1.35 (*m*), 2.28 (br. *d*, *J* = 12.8)	36.6 (CH_2)[d])	1.55–1.61 (*m*), 2.33 (br. *d*, *J* = 12.4)	37.2 (CH_2)
2	1.34–1.37 (*m*)	17.9 (CH_2)	1.75–1.80 (*m*)	18.2 (CH_2)
3	1.50 (br. *t*, *J* = 12.8), 1.40–1.45 (*m*)	36.1 (CH_2)	1.76–1.81 (*m*)	36.6 (CH_2)
4		45.7 (C)		46.3 (C)
5	2.35 (*dd*, *J* = 14.8, 2.0)	43.5 (CH)	2.67 (*d*, *J* = 14.0)	43.8 (CH)
6	2.31 (*dd*, *J* = 15.2, 14.8), 2.06 (br. *d*, *J* = 15.2)	37.1 (CH_2)	2.66 (*dd*, *J* = 16.0, 14.0), 2.48 (br. *d*, *J* = 16.0)	37.8 (CH_2)
7		196.0 (C)		198.9 (C)
8		122.8 (C)		131.4 (C)
9		145.7 (C)		147.8 (C)
10		36.8 (C)		37.0 (C)
11	6.75 (*s*)	108.6 (CH)	7.21 (*d*, *J* = 8.4)	125.0 (CH)
12		158.9 (C)	7.03 (*dd*, *J* = 8.4, 2.0)	122.1 (CH)
13		132.4 (C)		154.2 (C)
14	7.77 (*s*)	125.3 (CH)	7.45 (*d*, *J* = 2.0)	112.7 (CH)
15	3.17 (*sept.*, *J* = 7.0)	28.9 (CH)		
16	1.14 (*d*, *J* = 7.0)	22.0 (Me)		
17	1.15 (*d*, *J* = 7.0)	22.0 (Me)		
18		180.0 (C)		182.2 (C)
19	1.25 (*s*)	16.1 (Me)	1.31 (*s*)	16.2 (Me)
20	1.18 (*s*)	23.0 (Me)	1.25 (*s*)	23.8 (Me)

[a]) Recorded at 400 and 100 MHz, respectively. [b]) In (D_6)acetone. [c]) In $CDCl_3$. [d]) Attached H-atoms were assigned by DEPT experiments.

remaining two degrees must be due to two additional rings. The ^{13}C-NMR spectrum revealed the presence of a carboxylic acid (δ(C) 180.0 (*s*, C(18))), a conjugated ketone (δ(C) 196.0 (*s*, C(7))), and six aromatic C-atoms (δ(C) 108.6 (*d*, C(11)), 158.9 (*s*, C(12)), 145.7 (*s*, C(9)), 132.4 (*s*, C(13)), 125.3 (*d*, C(14)), and 122.8 (*s*, C(8))). Thus, compound **1** was tentatively proposed to be a 7-oxodehydroabietane derivative. These structural features were supported by the HMBCs (*Fig. 2*) H–C(5) (δ(H) 2.35)/C(3) (*t*, δ(C) 36.1), C(4) (*s*, δ(C) 45.7), C(7) (*s*, δ(C) 196.0), and C(9) (*s*, δ(C) 145.7); H–C(15) (δ(H) 3.17)/C(12) (*s*, δ(C) 158.9), C(13) (*s*, δ(C) 132.4), and C(14) (*d*, 125.3); H–C(11) (δ(H) 6.75)/C(10) (*s*, δ(C) 36.8); and H–C(14) (δ(H) 7.77)/C(7) (*s*, δ(C) 196.0) and C(8) (*s*, δ(C) 122.8). The relative configurations of the sterogenic centers in the tricyclic system were determined by significant NOE correlations (*Fig. 2*) H–C(5) (δ(H) 2.35)/H_α–C(6) (δ(H) 2.06); H_β–C(6) (δ(H) 2.31)/Me(19) (δ(H) 1.25), and Me(20) (δ(H) 1.18); and Me(19)/Me(20) in the NOESY spectrum (*Fig. 2*). Therefore, compound **1** was established as 12-hydroxy-7-oxoabieta-8,11,13-trien-18-oic acid, trivially named abietopinoic acid. The assignments of the H- and C-atoms were based on 2D-NMR and agreed well with the published values of similar partial structures [31][32], which were also supportive for the carboxylic acid group at C(18) in an

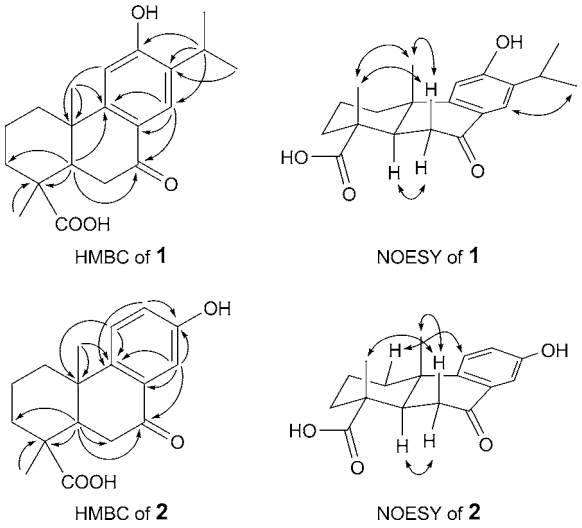

Fig. 2. *Selected HMBCs and NOESY correlations of compounds* **1** *and* **2**

equatorial orientation. The absolute configuration of compound **1** remains to be established (abietane or *ent*-abietane series).

The HR-EI-MS of **2** showed the molecular-ion peak at *m*/*z* 288.1357, which corresponded to the molecular formula, $C_{17}H_{20}O_4$, again indicating eight degrees of unsaturation. The IR spectrum displayed absorption bands for OH (3370 cm^{-1}), COOH (3300–2500, 1699 cm^{-1}), conjugated C=O (1679 cm^{-1}), and aromatic (1615, 1498 cm^{-1}) groups. The ^{1}H-NMR spectrum (*Table*) exhibited two *singlet*s of Me groups (δ(H) 1.25 (*s*, Me(20)), 1.31 (*s*, Me(19))) and an *ABX* system of an aromatic ring system (δ(H) 7.21 (*d*, J = 8.4, H–C(11)), 7.03 (*dd*, J = 8.4, 2.0, H–C(12)), and 7.45 (*d*, J = 2.0, H–C(14))). The H_β–C(1) signal at δ(H) 2.33 (br. *d*, J = 12.8) was in the characteristic region for dehydroabietane and dehydropodocarpane-type compounds [33][34]. Since compound **2** was a trinorditerpene (C_{17}), showing no evidence for the presence of an ^{i}Pr group in the NMR spectra, it was assumed to be most likely a dehydropodocarpane diterpenoid. The ^{13}C-NMR spectrum of **2** (*Table*) exhibited 17 C-atom signals, which were attributed, by DEPT experiments, to two Me and four aliphatic CH_2 groups, one aliphatic CH group, two aliphatic quaternary C-atoms, three olefinic CH groups, and three olefinic quaternary and two CO C-atoms. Among 17 ^{13}C-NMR signals, six belong to the phenyl C-atoms. The OH group was located at C(13) in the benzene ring, which was established by the HMBCs (*Fig. 2*) H–C(11) (δ(H) 7.21)/C(9) (*s*, δ(C) 147.8) and C(10) (*s*, δ(C) 37.0); H–C(12) (δ(H) 7.03)/C(9); and H–C(14) (δ(H) 7.45)/C(7) (*s*, δ(C) 198.9) and C(8) (*s*, δ(C) 131.4). The relative configuration of **2** was determined by phase-sensitive NOESY and molecular modeling. In the NOESY spectrum of **2**, the NOE correlations (*Fig. 2*) H_β–C(1) (δ(H) 2.33)/Me(20) (δ(H) 1.25) and H–C(11) (δ(H) 7.21; H_β–C(6) (δ(H) 2.66)/Me(20) and Me(19) (δ(H) 1.31); and H_α–C(6) (δ(H) 2.48)/H–C(5) (δ(H) 2.67) confirmed that both H–C(5) and COOH were α-oriented. Comparison of the ^{1}H- and ^{13}C-NMR data of compound **2** with those of 7-

oxodehydroabietic acid [24][35] indicated that **2** was oxidized at C(13). These evidences establish the structure of **2** as 13-hydroxy-7-oxopodocarpa-8,11,13-trien-18-oic acid, trivially named podopinoic acid.

The 7-deoxy analog, 13-hydroxypodocarpa-8,11,13-trien-18-oic acid, has been previously isolated from *P. massoniana* [24]. The observed upfield shift of C(19) is due to the two γ-gauche interactions with C(2) and C(6). This is consistent with an equatorial position of C(18)OOH and in agreement with previous reports. These results revealed that the genus *Pinus* may represent a good source of abietic acid diterpenes with an equatorial COOH group at C(4). Though podocarpene diterpenes do not occur abundantly in nature, this second report of an isolation of a secondary metabolite supports the proposed chemical taxonomy of the genus *Pinus* and is suggestive of a reasonable botanical classification of *P. massoniana.*

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Experimental Part

General. TLC: Silica gel *60 F_{254}* plates (*Merck*). Column chromatography (CC): SiO_2 (230–400 mesh ASTM, *Merck*). Semi-prep. HPLC: Normal phase column (*LiChrosorb Si 60*, 7 μm, 250 × 10 mm; *Merck & Co., Inc.*) on an *LDC Analytical-III* system. Optical rotations: *JASCO DIP-180* digital spectropolarimeter. UV Spectra: *Shimadzu UV-1601PC* spectrophotometer. IR Spectra: *Nicolet 510P* FT-IR spectrometer. NMR Spectra: *Varian Mercury plus 400* NMR spectrometer; chemical shifts δ, relative to the solvent resonance used as internal reference (TMS as standard) and were acquired in (D_6)acetone and $CDCl_3$. 2D-NMR Spectra: standard pulse sequences. HR-EI-MS: *JEOL SX-102A* mass spectrometer.

Plant Material. The heartwood of *P. massoniana* was collected at Luantashan, Nantau County, Taiwan, in December 1996. The plant material was identified by Prof. *Shao-Shun Ying*, Department of Forestry, National Taiwan University.

Extraction and Isolation. The air-dried sliced heartwood (7.4 kg) of *P. massoniana* were exhaustively extracted three times with acetone (40 l) at r.t. (7 d each). The combined MeOH extract was evaporated under reduced pressure at 45° to afford a brown residue, which was suspended in H_2O (3 l) and then partitioned sequentially, using AcOEt and BuOH (3 × 2 l) as solvent. The AcOEt fraction (135 g) was subjected to CC (SiO_2 (8 × 120 cm), gradient mixture hexane/AcOEt) to give ten fractions: *Fr. 1* (5000 ml; hexane/AcOEt 98:2), *Fr. 2* (4000 ml; hexane/AcOEt 95:5), *Fr. 3* (4000 ml; hexane/AcOEt 90:10), *Fr. 4* (4000 ml; hexane/AcOEt 80:20), *Fr. 5* (4000 ml; hexane/AcOEt 70:30), *Fr. 6* (4000 ml; hexane/AcOEt 60:40), *Fr. 7* (4000 ml; hexane/AcOEt 50:50), *Fr. 8* (3000 ml; hexane/AcOEt 30:70), *Fr. 9* (3000 ml; AcOEt), and *Fr. 10* (3000 ml; AcOEt/acetone 50:50). *Fr. 7* was further separated by CC (SiO_2 (5 × 50 cm); CH_2Cl_2/AcOEt (15:1 to 0:1) to afford six subfractions (each *ca.* 500 ml), *Frs. 7A–7F. Fr. 7D* was subjected to semiprep. HPLC (hexane/CH_2Cl_2/AcOEt 8:8:1) to yield **1** (6 mg) and **2** (16 mg).

12-Hydroxy-7-oxoabieta-8,11,13-trien-18-oic Acid (**1**). Colorless needles. M.p. 156–157°. $[\alpha]_D^{25} = +21.8$ ($c = 0.5$, $CHCl_3$). UV (MeOH): 232 (4.02), 281 (3.91), 302 (sh, 3.86). IR (KBr): 3334, 3300–2500, 1710, 1673, 1605, 1510. ^{1}H- and ^{13}C-NMR: see the *Table.* HR-EI-MS: 330.1837 (M^+, $C_{20}H_{26}O_4^+$; calc. 330.1831).

13-Hydroxy-7-oxopodocarpa-8,11,13-trien-18-oic Acid (**2**). Amorphous solid. $[\alpha]_D^{25} = +22.6$ ($c = 0.6$, $CHCl_3$). UV (MeOH): 222 (3.91), 255 (3.66), 324 (3.81). IR (KBr): 3370, 2925, 1699, 1679, 1615, 1498. ^{1}H- and ^{13}C-NMR: see the *Table.* HR-EI-MS: 288.1357 (M^+, $C_{17}H_{20}O_4^+$; calc. 288.1362).

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