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DITERPENOID ACIDS FROM *Pinus koraiensis*

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*Two new diterpenoid acids, pinusenocarp (**1**) and pinusenoid (**2**), were isolated from the pine cone of* Pinus koraiensis. *All the compounds were characterized on the basis of spectral analysis, viz. ^{1}H NMR, ^{13}C NMR, IR, UV, ESI-MS, and elemental analysis.*

Keywords: *Pinus koraiensis*, pinusenocarp, pinusenoid.

Pinus koraiensis is an economically important conifer and is indigenous to the northeast region of China [1]. Plants of the genus *Pinus* (Pinaceae) contain diterpenoids [2, 3], triterpenoids [4], flavonoids [5], and lignans [6]. Some of them are used in folk medicine as anti-asthmatic [7], anti-cancer [8], and anti-virus agents [9]. We have focused our attention on the biologically active compounds of the cones of coniferous trees treated as wastes in the forestry industry.

We have recently reported volatile oil [10] and diterpenes [11, 12] from the pine cone of the Pinaceae plant.

Herein we report the isolation of diterpene acids from this plant and their physicochemical properties.

Diterpene acids in alcohol (DAA) were first isolated from the raw material (4.5 kg) by ethanol extraction.

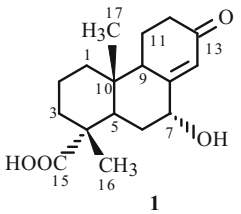
The EtOH fraction (780 g, 17.3%) dominated in the pine cone of *P. koraiensis*, which was separated by silica gel column chromatography eluted with petroleum ether (145 g), ethyl acetate (280 g), acetone (165 g), and methanol (82 g).

The EtOAc fraction (35.9%) dominated in the EtOH fraction, which was subjected to silica gel column chromatography eluted with CH_3Cl, CH_3Cl–MeOH (9:1; 8:2; 7:3; 1), and MeOH, and six fractions (A–F) were colleted on the basis of TLC.

During the investigation of the chemical constituents of this plant, two new diterpenoid acids from fraction D in the EtOAc extract of the cone of *P. koraiensis* have been isolated for the first time. In this paper, we present the isolation and structural elucidation of two new diterpenoid acids.

Column chromatography of various fractions of the ethanol extract isolated two new diterpenoid acids, which we called pinusenocarp (**1**) and pinusenoid (**2**).

Pinusenocarp (1). Elemental analysis agrees with the molecular formula $C_{17}H_{24}O_4$, mp 175–176°C. This molecular formula was confirmed by ESI-MS, *m/z* at 315 [M + Na]. The UV and IR spectra of **1** showed the presence of hydroxyl groups



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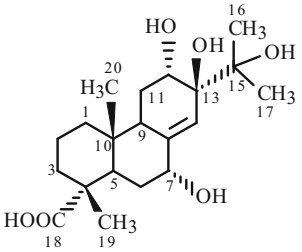

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(ν_{max} 3400 cm^{-1}), an α,β-unsaturated ketone (λ_{max} 235 nm; ν_{max} 1690 cm^{-1}), a trisubstituted double bond (ν_{max} 1612 and 833 cm^{-1}), and a carboxyl group (ν_{max} 2931 and 1718 cm^{-1}). The ^1H and ^{13}C NMR spectrum of **1** revealed signals for two tertiary methyl groups, six methylenes, two methines, two sp^3 quaternary carbons, an oxygenated methine (δ_H 4.16 ppm (1H, t, J = 3.0 Hz); δ_C 71.8 ppm), an α,β-unsaturated ketone [δ_H 6.05 ppm (1H, d, J = 2.0 Hz); δ_C 127.4, 164.7 and 202.5 ppm], and a carboxyl group (δ_C 183.2 ppm). Its structure was determined by a combination of HMQC and HMBC spectrum, which proved the existence of a podocarpane skeleton bearing a carboxyl group at C-4, a secondary hydroxyl group at C-7, and an α,β-unsaturated ketone at C-8(14)-13 positions in **1**. On the basis of the above spectral evidences, the structure of **1** was established to be 7α-hydroxypodocarp-8(14)-en-13-on-15-oic acid.

Pinusenoid (2). Elemental analysis agrees with the molecular formula $C_{20}H_{32}O_6$, mp 231–232°C. This molecular formula was confirmed by ESI-MS, *m/z* at 391 [M+Na]. The IR spectra of **2** showed the presence of hydroxyl groups (ν_{max} 3412 cm^{-1}), a trisubstituted double bond (ν_{max} 1610 and 830 cm^{-1}), and a carboxyl group (ν_{max} 2941 and 1720 cm^{-1}). The ^1H and ^{13}C NMR spectrum of **2** revealed signals for two tertiary methyl groups, a hydroxyisopropyl group, five methylenes, two methines, two sp^3 quaternary carbons, two secondary hydroxyl groups [δ_H 4.14 ppm (1H, t, J = 3.0 Hz) and 3.83 ppm (1H, br. s); δ_C 74.2 and 69.1 ppm], two tertiary hydroxyl groups (δ_C 71.9 and 76.1), a trisubstituted double bond [δ_H 6.12 ppm (1H, br. s); δ_C 145.0 and 128.6], and a carboxyl group (δ_C 181.1). From the above NMR data compound **2** was suggested to be a diterpenoid acid. Its structure was determined by a combination of HMQC and HMBC spectrum, which proved the existence of an abietane skeleton in **2**. The HMBC spectrum showed that the carboxyl group was located at C-18. The HMBC correlations between H-12 (H-14) and C-13 indicated that a tertiary hydroxyl group (δ_C 71.9 ppm) was located at C-13. The correlation between H-16 (H-17) and C-15 showed that the other tertiary hydroxyl group (δ_C 76.1 ppm) was connected to C-15. On the basis of the above spectral evidences, the structure of **2** was established to be 7α,12α,13β,15-tetrahydroxyabiet-8(14)-en-18-oic acid.

EXPERIMENTAL

Materials and Methods. UV spectra were obtained on a Shimadzu UV-260 spectrophotometer. IR spectra were obtained in KBr on a Perkin–Elmer 683 infrared spectrophotometer. ^1H and ^{13}C NMR spectra were recorded with a Inova-500 spectrometer operating at 500 MHz (^1H) and 125 MHz (^{13}C) using TMS as an internal standard. ESI-MS was obtained on an Autospec-UltimaETOF. Column chromatography was performed using silica gel (Qingdao Haiyang Chemical Group Co., Qing Dao, China), TLC was conducted on Si-gel GF_{254} (Qingdao Haiyang Chemical Group Co., Qing Dao, China) and monitored at UV 254 nm. Reversed Phase Si-gel (ODS, Merck) was purchased from Beijing Jinouya Chemical Company.

Plant Material. The pine cone of *P. koraiensis* was collected from Heilongjiang province of the People′s Republic of China in September 2005. A voucher specimen (No. 050916) has been deposited in the Herbarium of the School of Food Science and Engineering, Harbin Institute of Technology.

Extraction and Isolation. The cones of *P. koraiensis* (4.5 kg) were extracted three times with EtOH under reflux. The combined EtOH extract was concentrated to give a residue (780 g), which was separated by silica gel column chromatography eluted with petroleum ether, ethyl acetate, acetone, and methanol. The fractions were separately collected. The EtOAc fraction (280 g) was subjected to silica gel column chromatography eluted with CH_3Cl, CH_3Cl–MeOH (9:1; 8:2; 7:3; 1), and MeOH, and six fractions (A–F) were collected on the basis of TLC. Fraction D (45 g) was further subjected to silica gel column chromatography with petroleum ether–acetone (19:1; 9:1; 2) and acetone to yield a petroleum ether–acetone (9:1) eluate (2.15 g). The petroleum ether–acetone (9:1) eluate was subjected to reversed-phase silica gel (ODS) column chromatography with MeOH–H_2O (80:20) to obtain **1** (23 mg) and **2** (15 mg).

Pinusenocarp (1). (7α-Hydroxypodocarp-8(14)-en-13-on-15-oic acid), $C_{17}H_{24}O_4$, mp 175–176°C, $[\alpha]_D^{25}$ –47.5° (*c* 0.25, MeOH); UV (MeOH, λ_{max}, nm): 235 (log 4.02); IR (KBr, ν_{max}, cm^{-1}): 3400, 2931, 1718, 1690, 1612, 833; ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 1.16 (1H, m, H-1α), 1.75 (1H, m, H-1β), 1.58 (2H, m, H-2), 1.85 (1H, m, H-3α), 1.60 (1H, m, H-3β), 2.42 (1H, dd, J = 12.5, 2.5, H-5α), 1.48 (1H, ddd, J = 14.0, 2.5, 2.5, H-6α), 1.70 (1H, m, H-6β), 4.16 (1H, t, J = 3.0, H-7β), 2.51 (2H, ddd, J = 8.0, 5.5, 2.0, H-9α), 2.01 (1H, m, H-11α), 1.77 (1H, m, H-11β), 2.24 (1H, m, H-12α), 2.22 (1H, m, H-12β), 6.05 (1H, d, J = 3.0, H-14), 1.19 (3H, s, H-16), 0.86 (3H, s, H-17); ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 38.5 (C-1), 17.9 (C-2), 38.1 (C-3), 47.6 (C-4), 43.1 (C-5), 32.8 (C-6), 71.8 (C-7), 164.7 (C-8), 47.1 (C-9), 39.7 (C-10), 20.1 (C-11), 36.3 (C-12), 202.5 (C-13), 127.4 (C-14), 183.2 (C-15), 17.6 (C-16), 15.3 (C-17); ESI-MS, *m/z* at 315 $[M + Na]^+$.

Pinusenoid (2). (7α,12α,13β,15-tetrahydroxyabiet-8(14)-en-18-oic acid), $C_{20}H_{32}O_6$, mp 231–232°C, $[\alpha]_D^{25}$ –11.0° (*c* 0.35, MeOH); IR (KBr, ν_{max}, cm^{-1}): 3412, 2941, 1720, 1610, 830; ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 1.20 (1H, m, H-1α), 1.76 (1H, m, H-1β), 1.60 (2H, m, H-2), 1.88 (1H, m, H-3α), 1.62 (1H, m, H-3β), 2.40 (1H, dd, J = 13.5, 2.5, H-5α), 1.45 (1H, dt, J = 14.0, 2.5, H-6α), 1.72 (1H, m, H-6β), 4.14 (1H, t, J = 3.0, H-7β), 2.37 (2H, m, H-9α), 1.75 (1H, m, H-11α), 1.91 (1H, m, H-11β), 3.83 (1H, m, H-12β), 6.12 (1H, br.s, H-14), 1.28 (3H, s, H-16), 1.34 (3H, s, H-17), 1.17 (3H, s, H-19), 0.83 (3H, s, H-20); ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 39.9 (C-1), 18.9 (C-2), 38.7 (C-3), 47.9 (C-4), 43.8 (C-5), 33.1 (C-6), 74.2 (C-7), 145.0 (C-8), 44.2 (C-9), 39.0 (C-10), 22.5 (C-11), 69.1 (C-12), 71.9 (C-13), 128.6 (C-14), 76.1 (C-15), 23.1 (C-16), 24.3 (C-17), 181.1 (C-18), 16.9 (C-19), 15.0 (C-20); ESI-MS, *m/z* at 391 $[M + Na]^+$.

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REFERENCES

1. X. Yang, Y. C. Zhang, H. Zhang, and J. Wang, *J. Chin. Med. Mater.*, **31**, 53 (2008).
2. H. T. A. Cheung, T. Myase, M. P. Lenguyen, and M. A. Smal, *Tetrahedron*, **49**, 7903 (1993).
3. J. M. Fang, C. I. Lang, W. Lien, and Y. S. Cheng, *Phytochemistry*, **30**, 2793 (1991).
4. J. M. Fang, W. Y. Tsai, and Y. S. Cheng, *Phytochemistry*, **30**, 1333 (1991).
5. J. M. Fang, W. C. Su, and Y. S. Cheng, *Phytochemistry*, **27**, 1395 (1988).
6. W. S. Feng, Y. Z. Wang, X. K. Zheng, and Y. F. Bi, *Acta Pharm. Sinica*, **37**, 626 (2002).
7. L. F. Li, K. L. Shi, B. Y. Liu, Y. Y. Li, S. H. Wang, and Y. R. Feng, *J. Qiqihar Med. College*, **22**, 609 (2001).
8. H. Sakagami, K. Konno, T. Sato, N. Osawa, M. Fujimaki, and N. Komatsu, *Anticancer Res.*, **8**, 581 (1988).
9. K. P. Lai, J. Donovan, and H. Takayama, *AIDS Res. Hum. Retrovir.*, **6**, 205 (1990).
10. X. Y. Su, J. Wang, X. Yang, and W. Q. Cao, *Chin. J. Anal. Chem.*, **34**, 217 (2006).
11. X. Yang, Y. Ding, Z. H. Sun, and D. M. Zhang, *Acta Pharm. Sinica*, **40**, 435 (2005).
12. X. Yang, H. Zhang, Y. C. Zhang, Y. Ma, and J. Wang, *Fitoterapia*, **79**, 179 (2008).