A New Triterpenoid from *Crataegus cuneata*

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A new triterpenoid named cuneataol was isolated from the fruit of *Crataegus cuneata* SIEB. *et* ZUCC. On the basis of spectroscopic analysis, the structure was elucidated as 2,25-epoxy-2α,3β,19α-trihydroxyurs-12-en-28-oic acid.

Key words *Crataegus cuneata*; triterpenoid; hawthorn fruit; Rosaceae; *Crataegi Fructus*

Crataegi Fructus (Rosaceae) is the fruit of *Crataegus cuneata* SIEB. *et* ZUCC.: the English name is hawthorn fruit, which is known as "Nan Shanzha (Southern Hawthorn Fruit)". It has digestant action and is particularly useful for treating the stagnation of undigested meat and diarrhea with inadequate discharge from the bowels.[1] The hawthorn fruit from *C. oxyacantha* has shown hypocholesterolemic and antiatherosclerotic activity,[2] inhibits thromboxane A_2 biosynthesis,[3] and is used to treat cardiovascular disorders.[4] In addition, catecholamines,[5] flavonoids,[6] and procyanidines[7] were reported as known compounds from *C. oxyacantha*. However, a search of the literature has revealed the absence of any chemical work on *C. cuneata*. In our continuing chemical and biological investigations of natural medicines, we herein report the isolation and structural elucidation of a new triterpenoid called cuneataol from the fruit of *C. cuneata.*

The fruit of *C. cuneata* was extracted with MeOH, and the methanol extract (331.1 g) was subjected to Diaion HP-20 column chromatography ($H_2O \rightarrow$MeOH) to give seven fractions. Fraction 5 (eluted with MeOH, 9.0 g) was further purified by Sephadex LH-20 column chromatography (MeOH : $CHCl_3$ = 9 : 1) to give four fractions. Subsequent chromatography of fraction 3 (3.6 g) using silica gel (*n*-hexane : acetone = 1 : 1, $CHCl_3$: MeOH = 20 : 1) provided **1** (44.3 mg) as a white amorphous powder, showing $[\alpha]_D$ +32.1° (MeOH). A peak due to $[M]^+$ at *m*/*z* 502 was observed in the electron impact mass spectrum (EI-MS). The high resolution EI-MS of **1** displayed a molecular ion at *m*/*z* 502.3298 consisting of a molecular formula $C_{30}H_{46}O_6$. Its IR spectrum showed absorption due to a hydroxyl (3440 cm^{-1}) and a carboxyl (1693 cm^{-1}) group.

The ^{1}H-NMR spectrum exhibited signals due to the protons of six methyl groups at δ 0.92 (3H, s), 1.11 (3H, d, *J* = 6.7 Hz), 1.25 (3H, s), 1.37 (3H, s), 1.40 (3H, s), 1.70 (3H, s) and one singlet methine group δ 3.03 (1H, s), one oxygen-bearing methine group at δ 3.87 (1H, s), one hydroxyl methyl group at δ 4.00 (1H, d, *J* = 8.5 Hz), 4.11 (1H, d, *J* = 8.5 Hz), and one olefinic proton at δ 5.53 (1H, br s) (Table 1).

The ^{13}C-NMR [complete decoupling (COM), distortionless enhancement by polarization transfer (DEPT)] spectrum indicated signals due to one carboxylic acid carbon at δ 180.6, one pair of trisubstituted double bond carbons at δ 127.5 and 139.8, one acetal carbon at δ 106.5, one oxygen-bearing methine carbon at δ 81.2, one oxygen-bearing quaternary carbon at δ 72.6, one hydroxyl methyl carbon at δ 66.5, five quaternary carbons at δ 38.7, 39.7, 41.6, 48.4, 48.5, four methine carbons δ 40.2, 42.3, 51.3, 54.8, eight methylene carbons δ 20.7, 23.8, 26.4, 26.9, 29.5, 32.8, 38.2, 41.7, and six methyl carbons δ 16.7, 16.8, 23.9, 25.4, 27.0, 28.7 (Table 1). When the ^{1}H- and ^{13}C-NMR data of **1** were compared with those of rotundic acid (3β,19,23-trihydroxyurs-12-ene-oic acid),[8] they were in good agreement, except for those of the A, B ring. It suggested that **1** had an urs-12-ene triterpene system hydroxylated at C-19. The heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) experiments made assignments of the respective proton and carbon signals, as shown in Fig. 1. Especially, the correlations of H-3→C-1/C-2/C-4/C-5/C-23/C-24; H-9→C-5/C-10/C-25/C-26; H-12→C-14/C-18; H-18→C-13/C-14/C-17/C-19/C-20/C-28; H-23→C-4/C-24; H-24→C-4/C-23; H-25→C-1/C-2/C-5/C-10; H-26→C-8/C-9; H-27→C-8/C-13; H-29→C-18; H-30→C-

Table 1. ^{1}H- and ^{13}C-NMR Data for **1** in $CDCl_3$

C	δ_C	δ_H
C-1	41.7	2.21 (d, *J* = 8.5 Hz), 2.35 (d, *J* = 8.5 Hz)
C-2	106.5	
C-3	81.2	3.87 (s)
C-4	38.7	
C-5	51.3	1.50 (m)
C-6	20.7	1.48 (m), 1.57 (m)
C-7	32.8	1.44 (m), 1.67 (m)
C-8	39.7	
C-9	40.2	2.34 (m)
C-10	48.5	
C-11	23.8	2.04 (m), 2.26 (m)
C-12	127.5	5.53 (br s)
C-13	139.8	
C-14	41.6	
C-15	29.5	1.25 (m), 2.22 (m)
C-16	26.4	2.01 (m), 2.07 (dd, *J* = 6.0, 14.7 Hz)
C-17	48.4	
C-18	54.8	3.03 (s)
C-19	72.6	
C-20	42.3	1.49 (m)
C-21	26.9	1.33 (m), 1.53 (m)
C-22	38.2	2.02 (m), 2.14 (m)
C-23	28.7	1.37 (s)
C-24	25.4	1.25 (s)
C-25	66.5	4.00 (d, *J* = 8.5 Hz), 4.11 (d, *J* = 8.5 Hz)
C-26	16.8	0.92 (s)
C-27	23.9	1.70 (s)
C-28	180.6	
C-29	27.0	1.40 (s)
C-30	16.7	1.11 (d, *J* = 6.7 Hz)

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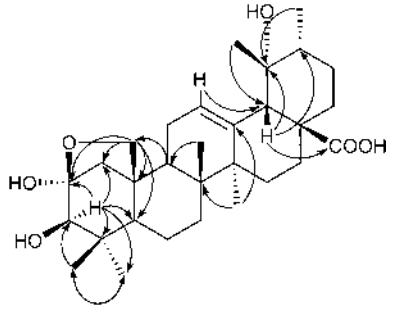

Fig. 1. HMBC (H to C) of **1**

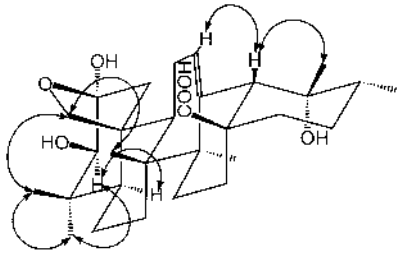

Fig. 2. NOESY Correlations of **1**

19 suggested the presence of dihydroxyl groups at C-3 and C-19, a hemiacetal group between C-2 and C-25, a carboxylic acid at C-28, and a secondary methyl group at C-20, thus constructing the plain structure of 2,25-epoxy-2,3,19-trihydroxyl ursolic acid derivative for compound **1**. A nuclear Overhauser and exchange spectroscopy (NOESY) between H-3 and H-5 or H-23 suggested the configuration of a 3β hydroxide, and between H-18 and H-29 suggested the configuration of a 19α hydroxide. A hemiacetal bond is formed between C-2 and C-25, so that the H-2 hydroxyl group has an α configuration. Therefore, the structure of the new triterpenoid **1** was determined to be a 2,25-epoxy-2α,3β,19α-trihydroxyurs-12-en-28-oic acid, and was termed cuneataol.

Experimental

The optical rotations were measured on a JASCO DIP-360 automatic digital polarimeter. The NMR spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C on a JEOL α-500 spectrometer, and chemical shifts were given on a δ (ppm) scale with tetramethylsilane as an internal standard. Standard pulse sequences were employed for the DEPT, HMQC, and HMBC experiments. NOESY spectra were measured with mixing times of 600 ms. EI-MS was measured on a JEOL DX-303HF spectrometer. TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck). Column chromatography was carried out on Kieselgel 60 (70—230 mesh and 230—400 mesh), MCI gel CHP-20P (Mitsubishi Chemical, Ind.), Sephadex LH-20 (Pharmacia), and Chromatorex ODS-DU 3050MT (Fuji Silysia).

Extraction and Isolation The dried powder of the fruit of *Crataegus cuneata* Sieb. *et* Zucc. (1.0 kg) was purchased from the Province of Liaoning Institute of Fruit Technology, and was extracted with MeOH twice under reflux. The combined extract (331.1 g) was concentrated and subjected to Diaion HP-20 column chromatography using $H_2O \rightarrow 100\%$ MeOH to give fractions 1 through 7. Fraction 5 (9.0 g) was separated by Sephadex LH-20 (MeOH : $CHCl_3$ = 9 : 1) and partitioned between AcOEt and 40% MeOH. The 40% MeOH extract (3.3 g) was further separated with silica gel [Hexane–Acetone (1 : 1), $CHCl_3$–MeOH (20 : 1)] to provide cuneataol (**1**) (44.3 mg).

Cuneataol (**1**): A white amorphous powder, $[\alpha]_D^{30}$ +32.1° (c=0.10, MeOH). IR (KBr) cm^{-1}: 3440, 1693. HR EI-MS m/z: 502.3298 $[M]^+$ (Calcd for $C_{30}H_{46}O_6$: 502.3294). EI-MS m/z: 502 $[M]^+$. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: Table 1. ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: Table 1.

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