สารประกอบไตรเทอร์พีนจากรากพุทราไทย

TRITERPENES FROM THE ROOT OF THAI ZIZIPHUS MAURITIANA

Panomwan Panseeta, Sunit Suksamram
Department of Chemistry, Faculty of Science, Srinakharinwirot University, Sukhumvit 23, Bangkok.

บทคัดย่อ

การศึกษาองค์ประกอบทางเคมีจากรากพุทราไทย สามารถแยกสารประกอบไตรเทอร์พีน 7 ชนิด โดยเป็นไตรเทอร์พีน ชนิด ceanothane 4 ชนิด : zizyberenalic acid, ceanothic acid, epiceanothic acid และ 24-hydroxyceanothic acid และเป็นไตรเทอร์พีน ชนิด lupane 3 ชนิด : lupeol, betulin และ betulinic acid โครงสร้าง และสเตอริโอเคมีของสารทราบได้จากการวิเคราะห์ข้อมูลทางสเปกโทรสโคปี โดยใช้เทคนิค NMR เป็นส่วนใหญ่ และโดยการเปรียบเทียบข้อมูลกับสารประกอบอื่นที่มีรายงานไว้แล้ว งานวิจัยนี้เป็นครั้งแรกของรายงานการพบสารประกอบไตรเทอร์พีนชนิด ceanothane ในพุทรา

คำสำคัญ: พุทรา, *Ziziphus mauritiana*, Rhamnaceae, ไตรเทอร์พีน

Abstract

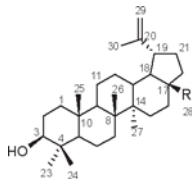
From the dried root of Thai *Ziziphus mauritiana* Lam. (Rhamnaceae), four ceanothane-type: zizyberenalic acid, ceanothic acid, epiceanothic acid and 24-hydroxyceanothic acid, together with three lupane-type triterpenes: lupeol, betulin and betulinic acid were isolated. Their structures were elucidated and stereochemical assignments were performed by extensive NMR spectroscopic data analysis and by comparison of their physical data with the reported values. This is the first report on the isolation of the ceanothane-type triterpenes from this plant species.

Keywords: *Ziziphus mauritiana*, Rhamnaceae, triterpene

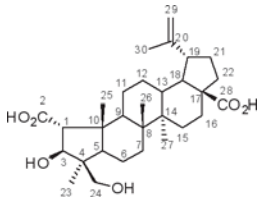
Introduction

Ziziphus mauritiana Lam. (or Phut-sa, in Thai) belongs to the Rhamnaceae family. This plant species is native to Thailand and Asian countries and has been used as traditional medicine. In Thailand, the fruit of *Z. mauritiana* has been used as laxative, expectorant and febrifuge [1], the stem bark and leaf decoction are used for treatment of diarrhea, ulcers, vomiting and indigestion [2]. In Myanmar, fruit is used for anticough, the root is used for antipyretic and leaf is a poultice for skin.

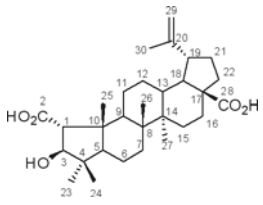
In traditional medicine of Korea, the seed is used as sedative [3]. Previous phytochemicals studies revealed that triterpenes and cyclopeptide alkaloids are major metabolites of this plant [4-5]. Triterpenoids obtained from *Z. mauritiana* displayed interesting biological properties, such as, tumor growth inhibitor [4], antiviral [6] and antibacterial activities [7]. Our works on Thai *Ziziphus* species have revealed some new bioactive compounds [7-8]. In a continuation of the search for bioactive compounds from Thai *Zizphus* plants, the EtOAc extract of *Z. mauritiana* root was investigated and this led to isolation of three lupane-type triterpenes : lupeol (**1**), betulin (**2**) and betulinic acid (**3**), in addition to, four ceanothane-type triterpenes : zizyberanalic acid (**4**), ceanothic acid (**5**), epiceanothic acid (**6**) and 24-hydroxyceanothic acid (**7**). This is the first report on isolation of the ceanothane-type triterpenes from this plant species.



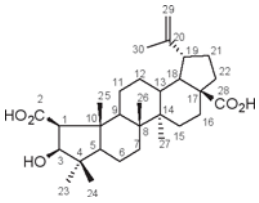
Lupeol (**1**) : R = CH_3
Betulin (**2**) : R = CH_2OH
Betulinic acid (**3**) : R = COOH



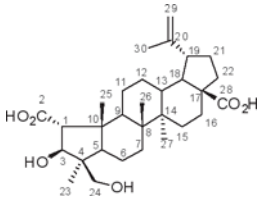
Zizyberenalic acid (**4**)



Ceanothic acid (**5**)



Epiceanothic acid (**6**)



24-Hydroxyceanothic acid (**7**)

Aims

To isolate, purify and structural elucidations of triterpenoids from Thai *Z. mauritiana* root.

Materials and methods

Plant Material

The root of *Z. mauritiana* was collected from Samchuk District, Suphanburi Province, Thailand, in June 2005 and a voucher specimen has been deposited at the Faculty of Science, Ramkhamhaeng University, Thailand.

General Experimental Procedures

Optical rotations were measured on a Perkin-Elmer 341 digital polarimeter. Melting points were determined using a Griffin melting point apparatus. UV spectra were obtained on a Shimadzu UV-2401 PC spectrophotometer.

IR spectra were recorded on a Perkin Elmer FT-IR Spectrum BX spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 300 FT-NMR spectrometer with $CDCl_3$ as solvent, operating at 300 MHz (^{1}H) and 75 MHz (^{13}C). For the spectra taken in $CDCl_3$ and C_5D_5N, the residual nondeuterated solvent signals at δ 7.24 and 8.71 and the solvent signals at δ 77.00 and 149.9 were used as references for ^{1}H and ^{13}C NMR spectra, respectively. Mass spectra were run on a Thermo Finnigan LC-Q mass spectrometer. Column chromatography was performed on Merck silica gel 60 (finner than 0.063 mm) and Sephadex LH-20. TLC was carried out using Merck precoated silica gel 60 F_{254} plates and spots on TLC were visualized under UV light and by spraying with anisaldehyde-H_2SO_4 followed by heating.

Extraction and Separation

The pulverized, dry root (4.5 kg) of *Z. mauritiana* was extracted successively with EtOAc (10 L X 3) and then with MeOH (10 L X 3) at room temperature for each one week and the solvents were evaporated to yield the EtOAc (29.5 g) and MeOH (45.6 g) extracts, respectively. A portion of the EtOAc extract (25.0 g) was fractionated by quick column chromatography (silica gel 60 GF_{254}), eluting with a gradient system with increasing amounts of the more polar solvent. The eluates were examined by TLC and 11 combined fractions (Fr.1-11) were obtained. Two successive column chromatography (silica gel), eluted with *n*-hexane-EtOAc, of fraction 5 (0.52 g) gave lupeol (**1**) as colorless solid (26.9 mg). Fraction 8 (2.59 g) was rechromatographed over silica gel with *n*-hexane-EtOAc as eluting solvent to give seven subfractions. Repeated column chromatography twice of subfraction 3 (777.2 mg) using *n*-hexane-CH_2Cl_2, CH_2Cl_2 and CH_2Cl_2-EtOAc as eluting solvent, yielded betulin (**2**) (37.1 mg) and zizyberenalic acid (**4**) (3.0 mg) as colorless solid. Fraction 9 (5.98 g) was recrystallized with MeOH to give the major compound **3** (2.38 g) as a colorless solid. Fraction 10 (8.83 g) was chromatographed over silica gel with CH_2Cl_2-MeOH as eluting solvent to give eight subfractions. Column chromatography (silica gel), eluted with *n*-hexane-CH_2Cl_2-EtOAc with increasing polarity, of subfraction 4 (2.19 g) afforded 11 subfractions (4a-4k). Ceanothic acid (**5**) (48.0 mg) was obtained as a colorless solid after recolumn chromatographed on silica gel of fraction 4f eluting with CH_2Cl_2-MeOH (1% increment of MeOH). Further column chromatography (silica gel) of fraction 4h (211.5 mg) employing solvent gradient CH_2Cl_2-EtOAC, furnished six fractions (fr. 4h-1 - 4h-6). Fraction 4h-5 (69.9 mg) was further separated by column chromatography (silica gel 60 RP-18) and eluted with H_2O-MeOH of decreasing polarity (5% increment of MeOH) to give **6** (17.6 mg) and **7** (19.2 mg) as colorless solid.

Results

The EtOAc extract of the pulverized, dried root of Thai *Z. mauritiana*, The EtOAc extract,

which gave mainly violet and blue colorations with anisaldehyde reagent, was subjected to further chromatographic isolation and purification. was subjected to extensive chromatographic isolation and purification and seven triterpenes, **1**-**7**, were obtained.

Lupeol (**1**): Colorless solid (26.9 mg, soluble in CH_2Cl_2); mp : 198-200 °C; R_f: 0.22 (8% EtOAc-Hexane), a violet coloration with anisaldehyde-H_2SO_4 reagent; IR ν_{max}^{KBr} cm^{-1}: 3342, 2944, 2867, 1638, 1465, 1453, 1380, 1043, 880; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.65 (1H, br *s*, H-29), 4.53 (1H, br *s*, H-29), 3.16 (1H, *dd*, *J* = 10.8, 5.2 Hz, H-3), 2.34 (1H, *d*, *J* = 11.0, 5.6 Hz, H-19), 1.65 (3H, *s*, H-30), 1.00 (3H, *s*, H-26), 0.93 (3H, *s*, H-23), 0.88 (3H, *s*, H-27), 0.80 (3H, *s*, H-25), 0.76 (3H, *s*, H-28), 0.73 (3H, *s*, H-24), 0.65 (1H, *d*, *J* = 8.9 Hz, H-5).

Betulin (**2**): Colorless solid (37.1 mg, soluble in CH_2Cl_2); mp : 236-237 °C (d); R_f: 0.22 (20% EtOAc-Hexane), a violet coloration with anisaldehyde-H_2SO_4 reagent; IR ν_{max}^{KBr} cm^{-1}: 3380, 2945, 2875, 1644, 1438, 1425, 1375, 1037, 1009, 879; ^{1}H NMR ($CDCl_3$, 300 MHz) δ4.65 (1H, br *s*, H-29), 4.55 (1H, br *s*, H-29), 3.77 (1H, *d*, *J* = 10.4 Hz, H-28), 3.30 (1H, *d*, *J* = 10.4 Hz, H-28), 3.16 (1H, *dd*, *J* = 10.5, 4.6 Hz, H-3), 2.34 (1H, *m*, H-19), 1.65 (3H, *s*, H-30), 0.99 (3H, *s*, H-26), 0.94 (3H, *s*, H-23), 0.94 (3H, *s*, H-27), 0.79 (3H, *s*, H-25), 0.73 (3H, *s*, H-24), 0.65 (1H, *d*, *J* = 8.8 Hz, H-5).

Betulinic acid (**3**): Colorless solid (2.38 g, soluble in CH_2Cl_2); mp : 280-282 °C (d); R_f : 0.36 (2% MeOH-CH_2Cl_2), a violet coloration with anisaldehyde-H_2SO_4 reagent; IR ν_{max}^{KBr} cm^{-1}: 3448, 2941, 2867, 1686, 1641, 1450, 1236, 1043, 885; ^{1}H NMR ($CDCl_3$, 300 MHz) δ4.71 (1H, br *s*, H-29), 4.58 (1H, br *s*, H-29), 3.16 (1H, *dd*, *J* = 10.9, 4.9 Hz, H-3), 2.98 (1H, *m*, H-19), 1.66 (3H, *s*, H-30), 0.95 (3H, *s*, H-27), 0.94 (3H, *s*, H-23), 0.90 (3H, *s*, H-26), 0.79 (3H, *s*, H-25), 0.72 (3H, *s*, H-24), 0.65 (1H, *d*, *J* = 8.2 Hz, H-5); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 180.3 (C-28), 150.3 (C-20), 109.6 (C-29), 79.0 (C-3), 55.3 (C-5), 37.1 (C-10), 27.9 (C-23), 19.3 (C-30), 16.1 (C-25), 16.0 (C-26), 15.3 (C-24), 14.6 (C-27).

Zizyberenalic acid (**4**): Colorless solid (3.0 mg, soluble in CH_2Cl_2); R_f: 0.37 (20% EtOAc-Hexane), a purple coloration with anisaldehyde-H_2SO_4 reagent; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.68 (1H, *s*, H-2), 6.54 (1H, *s*, H-3), 4.73 (1H, br *s*, H-29), 4.60 (1H, br *s*, H-29), 2.99 (1H, *dt*, *J* = 11.0, 4.6 Hz, H-13), 2.36 (1H, br *t*, *J* = 7.4 Hz, H-13), 2.28 (1H, br *d*, *J* = 12.0 Hz, H-16), 1.67 (3H, *s*, H-30), 1.13 (3H, *s*, H-23), 0.98 (3H, *s*, H-24), 1.12 (3H, *s*, H-25), 0.97 (3H, *s*, H-26), 0.97 (3H, *s*, H-27); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 191.4 (C-2), 181.9 (C-28), 163.3 (C-3), 157.3 (C-1), 150.0 (C-20), 109.9 (C-29), 63.0 (C-5), 52.1 (C-10), 28.1 (C-23), 20.4 (C-24), 19.2

(C-30), 19.0 (C-25), 16.8 (C-26) , 14.7 (C-27).

Ceanothic acid (**5**): Colorless solid (48.0 mg, soluble in CH_2Cl_2-MeOH); mp : 326-329 °C (d); R_f: 0.36 (2% MeOH-CH_2Cl_2), a violet coloration with anisaldehyde-H_2SO_4 reagent; IR ν_{max}^{KBr} cm^{-1}: 3478, 3067, 2939, 2867, 1693, 1640, 1455, 1376, 1312, 1206, 882; ^{1}H- and ^{13}C-NMR (C_5D_5N): see Table 1.

Epiceanothic acid (**6**): Colorless powder (17.6 mg, soluble in MeOH); mp : 260-261 °C (d); R_f: 0.37 (6% MeOH-CH_2Cl_2), a pale violet coloration with anisaldehyde-H_2SO_4 reagent; $[\alpha]_D^{25}$: -4.2 (c 0.35, MeOH); UV λ_{max}^{MeOH} nm (log ε) : 244 (2.81), 250 (2.89), 255 (2.92), 261 (2.79); IR ν_{max}^{KBr} cm^{-1}: 3505, 3409, 3077, 2955, 1706, 1642, 1463, 1377, 1238, 1207, 1188, 1058, 881; ESMS (+ve) *m/z* (% rel. intensity) : 995 $[2M+Na]^+$ (100), 487 $[M+H]^+$(1); ESMS (-ve) *m/z* (% rel. intensity) : 971 $[2M-H]^-$ (100), 485 $[M-H]^-$ (1); ^{1}H- and ^{13}C-NMR (C_5D_5N): see Table 1.

24-Hydroxyceanothic acid (**7**): Colorless solid (19.2 mg, soluble in MeOH); mp : 283-284 °C (d); R_f: 0.30 (6% MeOH-CH_2Cl_2), a blue coloration with anisaldehyde-H_2SO_4 reagent; $[\alpha]_D^{27}$ + 41.5 (c 0.30, MeOH); UV λ_{max}^{MeOH} nm (log ε) : 244 (2.84), 250 (2.92), 255 (2.96), 261 (2.82); ν^{KBr} cm^{-1}: 3461, 3077, 2941, 2870, 1689, 1646, 1450, 1377, 1210, 1035, 1019, 885; ESMS (+ve) *m/z* (% rel. intensity) : 503 $[M+H]^+$ (47); ESMS (-ve) *m/z* (% rel. intensity) : 501 $[M-H]^-$ (22) , 499 (100); ^{1}H- and ^{13}C-NMR (C_5D_5N): see Table 1.

Conclusion and discussion

From the EtOAc extract of dried root of Thai *Z. mauritiana*, three lupane-type triterpenes: lupeol (**1**), betulin (**2**) and betulinic acid (**3**), and four ceanothane-type triterpenes: zizyberenalic acid (**4**), ceanothic acid (**5**), epiceanothic acid (**6**) and 24-hydroxyceanothic acid (**7**) were isolated. Their structures were identified by means of spectroscopic analysis, mainly NMR, and by comparison their physical data with the reported values. Chromatographic comparison with authentic samples in several solvent systems was also used in this work.

Compounds **1-7,** which gave violet or blue coloration with anisaldehyde reagent, showed three characteristic singlet signals for isopropenyl group at around δ 4.65, 4.53 and 1.65 in ^{1}H NMR spectra. An observation of a double doublet methine proton resonance at around δ 3.16 (J = 10.8, 5.2 Hz) in ^{1}H NMR spectra ($CDCl_3$), suggesting that compounds **1-3** belong to the lupane-type triterpenes. Their ^{13}C NMR and DEPT spectra exhibited seven methyl singlets for **1**, six methyl singlets and an oxymethylene carbon signals (at δ_C 60.5) for **2** and six methyl singlets and a carboxyl carbon at (at δ_C 180.3) for **3**, in addition to a number of methine, methylene and quarternary carbon resonances. These observations together with the comparisons

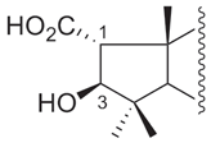
of their ^{1}H and ^{13}C NMR data with the reported values, led to the identification of compounds **1**-**3** as lupeol, betulin and betulinic acid, respectively. Compounds **1**, **2** and the major metabolite **3** are common triterpenes isolated previously from this plant species [4, 9].

Compound **4** was obtained as a minor colorless solid with the R_f value of 0.37 (20% EtOAc-Hexane). The ^{1}H and ^{13}C NMR spectra ($CDCl_3$, Table 1) displayed an isopropenyl group, five singlet methyls (d_H 0.97, 0.97, 0.98, 1.12, 1.13 and δ_C 14.7, 16.8, 20.4, 19.0, 28.1), an α,β-unsaturated aldehyde functional group [δ_H 9.68 (*s*), δ_C 191.4; δ_H 6.54 (*s*), δ_C 163.3] including a carboxyl carbon resonance (δ_C 181.9). Comparison of ^{13}C NMR spectral data of **4** with those of lupane acid [for example, betulinic acid (**3**)] and ceanothic acid (**5**) showed a similar pattern in the carbon resonances in rings B-E in these compounds but showed significant differences in the resonance signals for ring-A carbons. This indicated that **4** was a pentacyclic triterpene of the ceanothane series where ring A was five membered. Furthermore, the signals for the ring-junction carbons (C-5 at δ_C 63.0 and C-10 at δ 52.1) in **4** were shifted accordingly from the values of these carbons in betulinic acid (**3**) (C-5 at δ_C 55.3 and C-10 at δ 37.1) where ring A is six membered. Thus, compound **4** appeared to be zizyberenalic acid which contains an α,β-unsaturated aldehyde function on the five-membered ring A. The rest of the structure was the same as that of zizyberenalic acid (**4**) according to spectroscopic data [10] and chromatographic comparison of **4** with the authentic zizyberenalic acid in several solvent systems. Zizyberenalic acid (**4**) was found in the root of *Paliurus hemsleyanus* [10], the fruit of *Z. jujuba* Mill [11] and the root bark of *Z. cambodiana* [7].

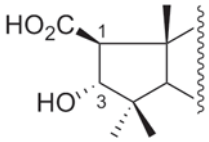
Compound **5** was obtained as a colorless solid with high polarity [R^f0.37 (6% MeOH-CH_2Cl_2)]. This compound exhibited IR absorption band for hydroxyl group (3478 cm^{-1}), carboxyl (1693 cm^{-1}) and olefinic double bond (C=CH_2, 1640 and 882 cm^{-1}). The ^{13}C NMR, DEPT, HMQC and COSY provided 30 carbon signals (including six methyls, nine methylenes, seven methines, six quaternary carbons and two carboxyl carbon signals) (C_5D_5N, Table 1). The ^{1}H NMR spectrum (C_5D_5N, Table 1) revealed an isopropenyl group, five singlet methyls (δ_H 1.05, 1.12, 1.26, 1.36 and 1.41), as well as the typical two correlated singlet methine protons at d_H 4.81 and 3.19 observed in the COSY spectrum suggesting that compound **5** could be ceanothic acid, a ceanothane-type triterpene with two carboxylic acid units at C-1 and C-17. In the HMBC spectrum, the singlet proton at δ_H 3.19 (H-1) showed correlations with C-2, C-3, C-4, C-5, C-10 and C-25, and a multiplet at δ_H 1.70 (H-18) showed correlations with C-13, C-14, C-17, C-19, C-20 and C-28 thus confirming the placement of the carboxyl groups at C-1 and C-17. The carbinol proton at C-3 also showed HMBC correlations with δ_C 178.2 (C-2), 43.9 (C-

4), 57.1 (C-5), 49.7 (C-10), 31.5 (C-23), 20.4 (C-24). Assignments of ^{1}H and ^{13}C NMR spectra data of **5** were confirmed by COSY, DEPT, HMQC and HMBC experiments. From the spectroscopic methods and chromatographic comparison with the authentic ceanothic acid in several solvent systems, the structure of compound **5** was hence assigned as ceanothic acid. Moreover, in the NOESY spectrum (Figure 1), the H-1 signal exhibited a cross-peak with CH_3-25, whist H-3 showed a cross-peak with CH_3-23. This evidence was consistent with the α-COOH and β-OH configurations of the 1- and 3-positions of ceanothic acid. Ceanothic acid (**5**) or 2α-carboxy-3β-hydroxy-A(1)-norlup-20(29)-en-28-oic acid has been isolated from the root of *Z. jujuba* Mill. var. *spinosa* [12], *Z. rugosa* [13], *Z. cambodiana* [7,14] and the root bark of *Ceanothus americanus* [15].

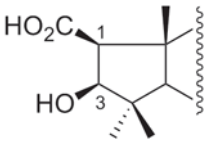
Compound **6** was obtained as a colorless powder with equal polarity [R_f 0.37 (6% MeOH-CH_2Cl_2)] as compound **5** and gave a pale violet coloration with anisaldehyde-H_2SO_4 reagent. Its IR spectrum showed absorption bands for hydroxyl group (3505 cm^{-1}), carboxyl (1706 cm^{-1}) and olefinic double bond (C=CH_2, 1642 and 881 cm^{-1}). It displayed a molecular ion at m/z 487 $[M+H]^+$ in the ESMS, which afforded the formula $C_{30}H_{46}O_5$. The ^{13}C NMR (Table 1) and DEPT spectra provided 30 carbon signals including six methyls, nine methylenes, seven methines, and eight quaternary carbons including two carboxylic acid carbon signals. The ^{1}H and ^{13}C NMR spectra (C_5D_5N, Table 1) of compound **6** showed a ceanothane type triterpene pattern for an isopropenyl group and five additional singlet methyl protons (δ_H 1.05, 1.12, 1.12, 1.20, 1.66 and d_C 14.7, 15.0, 17.0, 20.0, 32.2) with two carboxyls at d_C 176.2 and 179.0. The NMR data of **6** was very similar to that of ceanothic acid except for the presence of two doublets signals at δ_H 2.89 (J = 7.2 Hz) and 4.66 (J = 7.2 Hz) in the former instead of two broad singlets at δ_H 3.19 and 4.81 in the latter. In the HMBC spectrum (Figure 1), the proton at d_H 2.89 showed correlations with δ_C 176.2 (carboxylic carbon C-2), 83.1 (carbinol carbon C-3), 51.2 (C-9), 48.3 (C-10) and 14.7 (C-25), whilst the signal at d_H 4.66 showed connectivities with δ_C 176.2 (C-1), 48.3 (C-10), 32.2 (C-23) and 20.0 (C-24) suggesting that compound **6** could be an isomer of ceanothic acid (**5**). Thus the stereocenter at H-1 and H-3 in the ring A of **6** could be drawn as **5a**-**5d**,



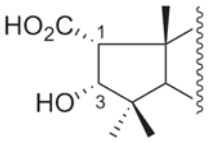
(**5a**) H-1β/H-3α
$J_{1,3}$ = 1.0 Hz [16]



(**5b**) H-1α/H-3β
$J_{1,3}$ = 9.0 Hz [16-17]



(**5c**) H-1α/H-3α
$J_{1,3}$ = 7.3 Hz [16,18]



(**5d**) H-1β/H-3β
$J_{1,3}$ = 7.0 Hz [16]

in which isomer **5a** was ceanothic acid (**5**). The isomer **5b** has been reported as isoceanothic acid, which isolated from the stem of *Z. xylopyrus* [17], and the $J_{1,3}$ value observed as 9.0 Hz [16]. The isomer **5c** has been isolated from the seed of *Z. jujuba* var. *spinosa* [18] as epiceanothic acid with the $J_{1,3}$ value of 7.3 Hz [16], whereas the isomer **5d** was a synthetic triterpene with the reported $J_{1,3}$ value of 7.0 Hz [16]. From the comparisons of ^{1}H and ^{13}C NMR data (Table 1) including the $J_{1,3}$ value for H-1 and H-3 protons of compound **6** with these four isomers, compound **6** could be of H-1α and H-3α in stereochemistry. NOE interactions also observed between H-1 with H-3 and CH_3-23, whereas CH_3-24 showed a cross-peak with CH_3-25 in the NOESY experiment, confirmed that H-1, H-3 and CH_3-23 resided on the same α-side as shown in Figure 1. Compound **6** displayed levorotatory optical rotation ($[\alpha]_D^{27}$-4.2) which comparable to that of epiceanothic acid (**6**) ($[\alpha]_D^{25}$ = -5.0) [18] and (H-1α/H-3α)-epiceanothic acid dimethyl ester ($[\alpha]_D^{20}$ = -9.0 [16]), whereas (H-1α/H-3α)-isoceanothic acid dimethyl ester ($[\alpha]_D^{20}$ = +37) [16] and isoceanothic acid ($[\alpha]_D$=+38.2) [17] showed dextrorotation. From the spectroscopic evidences and physical data, the structure of compound **6** was concluded to be 2β-carboxy-3β-hydroxy-A(1)-norlup-20(29)-en-28-oic acid or epiceanothic acid (**6**). Epiceanothic acid has been isolated from seed of *Z. jujuba* var. *spinosa* [18]. This is the first report of epiceanothic acid obtained from this plant.

Compound **7** was the most polar triterpene obtained from this plant with the R_f value of 0.30 (6% MeOH-CH_2Cl_2) as a colorless solid and gave a blue coloration with anisaldehyde-H_2SO_4 reagent. It exhibited IR absorption band for hydroxyl group (3461 cm^{-1}), carboxyl (1689 cm^{-1}) and olefinic double bond (C=CH_2, 1646 and 885 cm^{-1}). Its ESMS showed the molecular ion at m/z 503 $[M+H]^+$, corresponding to the formula $C_{30}H_{46}O_6$, which indicated the presence of one more oxygen atom than that of ceanothic acid. The ^{1}H NMR spectrum (Table 1) of compound **7** (in C_5H_5N) was similar to that of ceanothic acid (**5**) with only few differences were observed. The ^{1}H NMR spectrum of compound **7** displayed two singlet methine protons at δ_H 3.23 (H-1) and 4.92 (H-3), an AX methylene protons at δ_H 3.67 and 4.60 (J = 10.7), an isopropenyl group

(d_H 4.83, 4.64 and 1.63) and four additional singlet methyls (d_H 1.02, 1.08, 1.42 and 1.77), one less singlet methyl signal than that of ceanothic acid (**5**). In the ^{13}C NMR and DEPT spectra, 30 carbon signals were observed, including five methyls, 10 methylenes, seven methines, and eight quaternary carbons, as well as two carboxylic carbon signals (Table 1). The AX coupling system of methylene proton at δ_H 3.67 and 4.60 exhibited cross-peaks with C-3 (δ_C 85.8), C-4 (d_C 48.5) and C-5 (δ_C 57.2) in the HMBC spectrum (Figure 1). The hydroxylated position should therefore be at either C-23 or C-24. This difference was also observed in the ^{13}C NMR spectrum, in which a methyl signal at d_C 20.4 of ceanothic acid (**5**) was replaced by a hydroxylated methylene signal at δ_C 66.7, suggesting that compound **7** was a hydroxyceanothic acid. The location of this hydroxymethylene group was assigned by the observation of NOESY correlations (Figure 1). In the NOESY spectrum, the methyl signal proton at δ_H 1.42 (CH_3-25) showed correlations with protons at δ_H 3.23 (H-1) and 4.06 (CH_2-24b) indicating that they were on the same β-side. Similarly, NOE interactions were observed between CH_3-23 and H-3, and between CH_3-23 and CH_2-24a suggesting that they resided on the same α-side. Thus the relative stereochemistry of substituents on ring A of the ceanothane nucleus in compound **7** was shown in Figure 1 which was consistent with 24-hydroxyceanothic acid dimethyl ester, a compound isolated from the EtOH extract of the root of *Paliurus ramosissimus* (Rhamnaceae) [19]. It should be noted that NOE interaction was also observed between H-1 and H-3 but no NOE was observed between H-1 and H-24. This implied that H-1 and H-3 should reside in a suitable proximity (Figure 1). Comparison of the ^1H and ^{13}C NMR (recorded in 5% CD_3OD-$CDCl_3$) of compound **7** with those of 24-hydroxyceanothic acid dimethyl ester ($CDCl_3$), and the optical rotation of both compounds ($[\alpha]_D^{27}$ + 41.58 for compound **7** and $[\alpha]_D^{24}$ = +51.5 for 24-hydroxyceanothic acid dimethyl ester [19]), thus compound **7** should have the same stereostructure as that of 24-hydroxyceanothic acid dimethyl ester. From the spectroscopic evidence and the physical data, the structure of compound **7** was thus concluded to be 24-hydroxyceanothic acid. 24-Hydroxyceanothic acid (**7**) was isolated for the first time from this plant species.

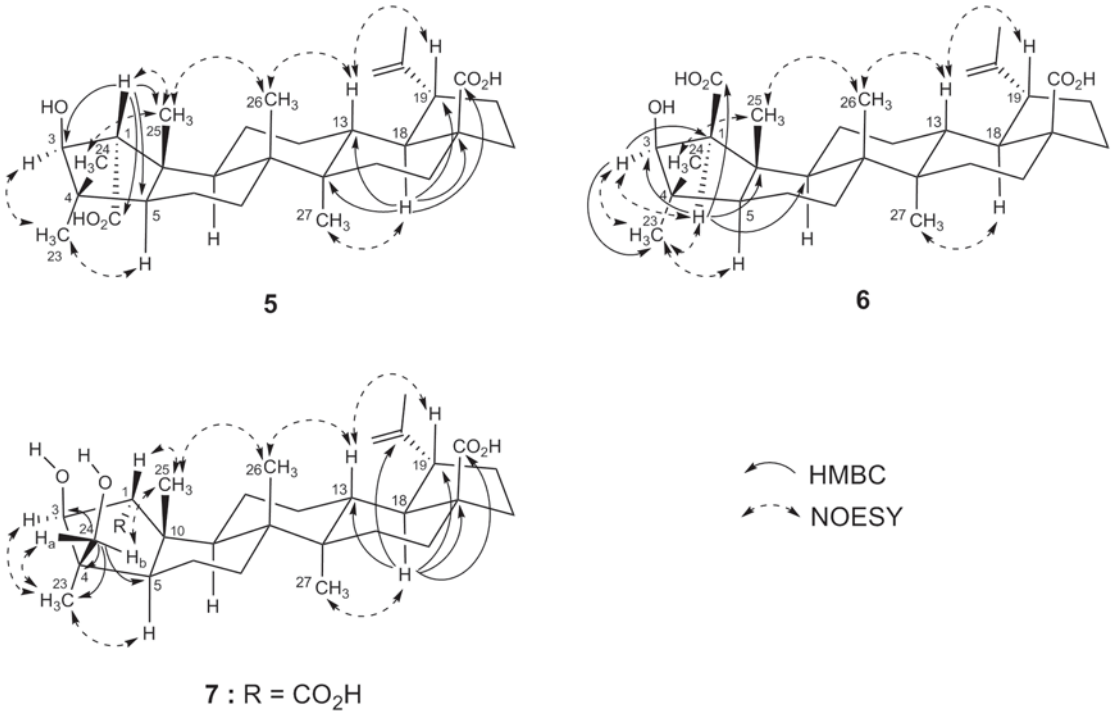


Figure 1 Selected HMBC and NOESY correlations of compounds **5-7**

Table 1 ^{1}H and ^{13}C NMR data of ceanothic acid (**5**), isoceanothic acid (**6**) and 24-hydroxyceanothic acid (**7**)in C_5D_5N

position	δ_H (mult., *J* in Hz)			δ_C		
	5	**6**	**7**	**5**	**6**	**7**
1	3.19 (1H, *s*)	2.89 (1H, *d*, *J* = 7.2)	3.23 (1H, *s*)	67.0	63.2	66.4
2				178.2	176.2	177.7
3	4.81 (1H, *s*)	4.66 (1H, *d*, *J* = 7.2)	4.92 (1H, *s*)	84.8	83.1	85.8
4				43.9	43.0	48.5
5	2.18 (1H, *m*)	1.74 (1H, *m*)	2.28 (1H, *m*)	57.1	62.7	57.2
6	1.47 (2H, *m*)	1.57 (1H, *m*)	1.51 (2H, *m*)	19.1	18.4	18.5
		1.35 (1H, *m*)				
7	1.57 (2H, *m*)	1.39 (2H, *m*)	1.45 (1H, *m*)	34.8	34.9	35.2
			1.31 (1H, *m*)			
8				43.6	42.0	43.5
9	2.18 (1H, *m*)	1.86 (1H, *m*)	2.12 (1H, *m*)	45.1	51.2	45.3
10				49.7	48.3	50.0
11	2.07 (1H, *m*)	2.00 (1H, *m*)	2.06 (1H, *m*)	24.3	24.6	24.3
	1.47 (1H, *m*)	1.74 (1H, *m*)	1.56 (1H, *m*)			
12	1.94 (1H, *m*)	1.94 (2H, *m*)	1.94 (1H, *m*)	26.3	25.9	26.2
	1.29 (1H, *m*)		1.32 (1H, *m*)			

position	δ_H (mult., *J* in Hz)			δ_C		
	5	6	7	5	6	7
13	2.75 (1H, *dt*, *J* = 12.7, 3.0)	2.75 (1H, br *t*, *J* = 10.8)	2.74 (1H, br *t*, *J* = 10.2)	39.2	38.6	39.1
14				42.2	43.1	42.0
15	1.84 (2H, *m*)	1.90 (1H, *m*) 1.24 (1H, *m*)	1.83 (1H, *m*) 1.17 (1H, *m*)	30.6	30.5	30.5
16	2.57 (2H, br *d*, *J* = 12.8)	2.61 (1H, br *d*, *J* = 12.8) 1.53 (1H, *m*)	2.57 (1H, br *d*, *J* = 12.8) 1.42 (1H, *m*)	33.0	33.0	33.0
17				56.7	56.6	56.7
18	1.70 (1H, *m*)	1.70 (1H, *m*)	1.63 (1H, *m*)	49.8	49.8	49.7
19	3.46 (1H, *m*)	3.46 (1H, *m*)	3.48 (1H, br *t*, *J* ca 10.7)	47.7	47.8	47.6
20				151.2	151.2	151.2
21	2.18 (1H, *m*) 1.18 (1H, *m*)	2.20 (1H, *m*) 1.50 (1H, *m*)	2.22 (1H, *m*) 1.48 (1H, *m*)	31.3	31.3	31.3
22	2.18 (1H, *m*) 1.49 (1H, *m*)	2.23 (1H, *m*) 1.48 (1H, *m*)	2.18 (1H, *m*) 1.48 (1H, *m*)	37.6	37.7	37.6
23	1.41 (3H, *s*)	1.12 (3H, *s*)	1.77 (3H, *s*)	31.5	32.2	25.7
24	1.26 (3H, *s*)	1.20 (3H, *s*)	4.60 (1H, *d*, *J* = 10.7, H_b) 3.67 (1H, *d*, *J* = 10.7, H_a)	20.4	20.0	66.7
25	1.36 (3H, *s*)	1.66 (3H, *s*)	1.42 (3H, *s*)	18.9	14.7	19.0
26	1.12 (3H, *s*)	1.12 (3H, *s*)	1.08 (3H, *s*)	17.1	17.0	17.1
27	1.05 (3H, *s*)	1.05 (3H, *s*)	1.02 (3H, *s*)	15.1	15.0	15.1
28				179.0	179.0	179.1
29	4.83 (1H, br *s*) 4.64 (1H, br *s*)	4.85 (1H, br *s*) 4.69 (1H, br *s*)	4.83 (1H, br *s*) 4.64 (1H, br *s*)	109.8	110.0	109.8
30	1.63 (1H, *s*)	1.74 (3H, *s*)	1.63 (3H, *s*)	19.6	19.5	19.6

Acknowledgements

This work was supported by The Thailand Research Fund and Srinakharinwirot University.

References

[1] พงษ์ศักดิ์ พลเสนา. (2550). *พืชสมุนไพรในสวนป่าสมุนไพรเขาหินซ้อน.* ปราจีนบุรี: เจตนารมณ์ภัณฑ์.

[2] Bunyapraphatsara, N. and Chokechaijaroenporn, O. (1999). In: Thai medicinal plants, vol. 3. Bangkok: Mahidol University and National Center for Genetic Engineering and Biotechnology.

[3] วิทย์ เที่ยงบูรณธรรม. (2542). *พจนานุกรมสมุนไพรไทย.* พิมพ์ครั้งที่ 5. กรุงเทพฯ: รวมสาส์น.

[4] Pezzuto, J. M., Dsagupta, T. K. and Kim, D. S. H. L. Use of betulinic acid derivatives for the treatment and prevention of melanoma [abstract]. PCT Int Appl, [online]. Available: SciFinder ScholarCAN 130:10621. 1998.

[5] Tschesche, R., Wilhelm, H., Kaussmann, E. U. and Eckhardt, G. (1974). Alkaloids from Rhamnaceae. XVII. Mauritine-C, -D, -E and -F, new peptide alkaloids from *Ziziphus mauritiana*. *Liebigs Annalen der Chemie*, 10: 1694-1701.

[6] Huang, R. L., Wang, W. Y., Kuo, Y. H. and Lin, Y. L. Cytotoxic triterpenes from the fruit of *Ziziphus jujuba* [abstract]. *Chinese Pharmaceutical Journal (Taiwan),* 53(4): 179-184. [online]. Available: SciFinder Scholar CAN 136:131533. 2001.

[7] Suksamrarn, S., Panseeta, P., Kunchanawatta, S., Distaporn, T., Ruktasing, S. and Suksamrarn, A. (2006). Ceanothane- and lupane-type triterpenes with antiplasmodial and antimycobacterial activities from *Ziziphus cambodiana*. *Chemical & Pharmaceutical Bulletin*, 54(4): 535-537.

[8] Suksamrarn, S., Suwannapoch, N., Aunchai, N., Kuno, M., Rananukul, P., Haritakun, R., Jansakul, C. and Ruchirawat, S. (2005). Ziziphine N, O, P and Q, new antiplasmodial cyclopeptide alkaloids from *Ziziphus oenoplia* var. *brunoniana*. *Tetrahedron*, 61: 1175-1180.

[9] Chauhan, J. S. and Srivastava, S. K. Chemical investigation of the stem of *Zizyphus mauritiana* (N.O. Rhamnaceae). *Proceedings of National Academy of Science, India*, Sect A, 48(1): 6. [online]. Available: SciFinder Scholar CAN 90:118114. 1978.

[10] Lee, S. S., Shy, S. N. and Liu, K. C. S. (1997). Triterpenes from *Paliurus hemsleyanus*. *Phytochemistry*, 46(3): 549-554.

[11] Lee, S. M., Park, J. G., Lee, Y. H., Lee, C. G., Min, B. S., Kim, J. H, et al. (2004). Anti-complementary activity of triterpenoides from fruits of *Zizyphus jujuba*. *Biological & Pharmaceutical Bulletin*, 27(11): 1883-1886.

[12] Lee, S. S., Lin, B. F., Chen, K. C. S. Chemical constituents from the roots of *Ziziphus jujuba* Mill. var. *spinosa* (Bunge) Hu.(II) [abstract]. *Chiness Pharmaceutical Journal (Taipei)*, 47(6): 511-519, [online]. Available: SciFinder Scholar CAN 124:337822. 1995.

[13] Netsawangwicha, J. (2005). A study on chemical constituents from the root barks of *Ziziphus rugosa* Lam. M.Sc. Thesis, Bangkok: Srinakharinwirot University, Thailand.

[14] Panseeta, P. (2004). Chemical constituents from the root barks of *Ziziphus cambodiana* Pierre. M.Ed.(Chemistry) Thesis, Bangkok: Srinakharinwirot University, Thailand.

[15] Li, X.-C., Cai, L. and Wu, C. D. (1997). Antimicrobial compounds from *Ceanothus americanus* against oral pathogens. *Phytochemistry*, 46(1): 97-102.

[16] Eade, R. A., Grant, P. K., McGrath, M. J. A., Simes, J. J. H. and Wootton, M. (1971). Extractives of Australian timbers XI. The stereochemistry of ceanothic acid. *Australian Journal of Chemistry*, 24: 621-632.

[17] Jagadeesh, S. G., Krupadanam, G. L. D. and Srimannarayana, G. (2000). A new triterpenoid from *Zizyphus xylopyrus* stem wood. *Indian Journal of Chemistry*, 39B, 396-398.

[18] Li, L. M., Liao, X., Peng, S. L. and Ding, L. S. (2005). Chemical constituents from the seeds of *Ziziphus jujuba* var. *spinosa* (Bunge) Hu. *Journal of Integrative Plant Biology*, 47(4): 494-498.

[19] Lee, S. S., Lin, C. J. and Liu, K. C. (1992). Two triterpenes from *Paliurus ramosissimus*. *Journal of Natural Products*, 55(5): 602-606.