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SHORT REPORTS

CHEMICAL CONSTITUENTS OF THE ROOTS OF *BRIDELIA TOMENTOSA* BL.

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ABSTRACT

Systematic fractionation of the chloroform extract of the roots of Bridelia tomentosa *Bl. led to the isolation of 24-methyllanosta-9(11),25-dien-3-one;24,24-dimethyllanosta-9(11),25-dien-3-one; friedelin; friedelan-3β-ol; β-sitosterol; stigmasterol; triacanthine; β-sitosteryl-3-O-β-D-glucopyranoside; and stigmasteryl-3-O-β-D-glucopyranoside.*

Bridelia tomentosa Bl. (syn. *Bridelia monoica* Merr.), a traditional medicinal plant in Thailand, locally known as "Khon non", is a small tree in the Euphorbiaceae family. A decoction of the bark or leaves is used for colic. A decoction of leaves with parts of other plants is used for high fever. The root serves as a medicine taken over the first three days after childbirth.[1]

Hui and Fung[2] investigated the leaves and stems of *B. monoica* Merr. and reported the isolation of friedelin, friedelan-3β-ol,glutin-5-ene-3β-ol , stigmasterol, β-sitosterol and a long chain aliphatic compound, $C_{20}H_{38}O_2$. Our work describes the extraction, isolation and identification of nine constituents of the roots of *B. tomentosa* Bl.

GENERAL EXPERIMENTAL METHODS

Melting points were determined by a Fisher-Johns melting point apparatus, and are uncorrected. The IR spectra were recorded with a Shimadzu Infrared Spectrophotometer IR-440 using KBr discs. ^1H NMR and ^{13}C NMR spectra were recorded on a Jeol JNM-FX 90 Q NMR spectrometer operating at 90 and 22.5 MHz respectively, unless otherwise indicated. The mass spectra were obtained on a Jeol JMS-DX 300 mass spectrometer. Elemental analyses were carried out on a Perkin-Elmer 240C elemental analyzer at the Science and Technology Research Equipment Center, Chulalongkorn University, Bangkok, Thailand.

Plant Materials

The dried roots of *B. tomentosa* Bl. were obtained from Rayong Province, Thailand in February 1986. The voucher specimen, BK 37631, has been deposited at the Herbarium of Botany Section, Botany and Weed Science Division, Department of Agriculture, Ministry of Agriculture and Cooperative, Bangkok, Thailand.

Extraction and Isolation

Dried and ground roots of *B. tomentosa* Bl. (4.9 kg) were exhaustively extracted with methanol by maceration. The methanolic extract was evaporated and partitioned with chloroform. The chloroform extract was concentrated to give a brown semisolid (50.7 g). The semisolid (42.1 g) was chromatographed on a silica gel column (510 g) and eluted successively with hexane, mixtures of hexane and chloroform with increasing amount of chloroform, chloroform, mixtures of chloroform and methanol with increasing amount of methanol. Successive fractions, based on TLC behavior, were combined, concentrated and purified as usual.

The first component was eluted with chloroform : hexane (1:3) as a white needle solid (680 mg), m.p. 150-151°C (from methanol). It appeared to be a mixture of two closely related triterpenoids. The GC-MS indicated that they were $C_{31}H_{50}O$ and $C_{32}H_{52}O$ compounds. The ^{1}H NMR (250 MHz) and ^{13}C NMR (62.5 MHz) indicated they were a mixture a mixture of a new 31-carbon triterpenoid; 24-methyllanosta-9(11), 25-dien-3-one (1A, 60%), and a known 32-carbon triterpenoid; 24,24-dimethyllanosta-9(11), 25-dien-3-one (1B, 40%)[3]

$[\alpha]^{25}_{D}$ + 63.7° (c = 1.0, $CHCl_3$);

Rf 0.7 (silica gel/chloroform)

UV λ_{max} ($CHCl_3$) 291 nm (ϵ33)

IR θ_{max} (KBr) 3060, 3040(C=CH), 1710(C=0), 1640, 880 cm^{-1}

MS, m/e (rel.intensity) 1A, 438(M^+,28), 423(79), 311(100), 257(44), 245(41); 1B, 452(M^+,20), 437(50), 395(9), 311(92), 257(38), 245(32), 55(100).

^{1}H NMR ($CDCl_3$, 250 MHz) δ(ppm) : 1A, 0.66s(H-18), 0.74s(H-28), 0.87d(6.3)(H-21), 1.00d(6.9)(H-31), 1.07s(H-29,H-30), 1.23s(H-19), 1.64brs(H-27), 1.92m, 2.08m(H-12), 2.1m (H-24), 2.10ddd(13.2,6.3,3.1) (H-1β), 2.23br d(12.3)(H-8), 2.40ddd(15.4,5.4,3.1)(H-2α), 2.72ddd (15.4, 13.3, 6.3)(H-2β), 4.67br s(H-26), 5.28 br d(5.9)(H-11); 1B (H-18, H-28, H-21), H-31, H-29, H-30, H-19, H-27, H-12, H-24, H-1β, H-8, H-2α, H-2β, H-11 same as 1A), 1.01s(H-31), 1.02s(H-32), 1.69br s(H-27), 4.67 br s, 4.72 br s(H-26)

^{13}C NMR ($CDCL_3$, 62.9 MHz) δ(ppm) : <u>1A</u>, 14.4(C-18), 18.4(C-28), 18.4(C-31), 18.6(C-21), 20.2(C-27), 21.8(C-19), 22.0(C-30), 22.5(C-7), 25.6(C-29), 27.7(C-6), 27.9(C-16), 31.4(C-22), 33.9(C-15), 33.9(C-23), 34.9(C-2), 36.0(C-20), 36.7(C-1), 37.1(C-12), 39.0(C-10), 41.6(C-24) 41.8(C-8), 44.2(C-13), 46.9(C-4), 47.7(C-14), 50.8(C-17), 53.4(C-5), 109.4(C-26), 116.3(C-11), 147.0(C-9), 150.1(C-25), 217.2(C-3); <u>1B</u>, (C-1 to C-16, C-18, C-19, C-28, C-29, C-30 same as <u>1A</u>), 18.5 (C-21), 19.4(C-27), 27.2(C-31), 27.5(C-32), 30.7(C-22), 36.6(C-20), 37.3(C-23), 38.7(C-24), 50.7(C-17), 109.3(C-26), 152.3(C-25)

Anal.Found : C, 85.09; H, 11.60. Calcd. for $C_{31}H_{50}O$: C, 84.87; H, 11.49
Calcd.for $C_{32}H_{52}O$: C, 84.89; H, 11.58

The second component was eluted with chloroform : hexane (1:3) as a white solid (200 mg), m.p. 246-248°C (from methanol-chloroform). This component gave pink color with Liebermann-Burchard reagent which indicated a triterpenoidal in nature. Thin-layer chromatography ($SiO_2/CHCl_3$) indicated two components with Rf value of 0.44 and 0.34. The two components were separated by preparative thin-layer chromatography and after recrystallization friedelin (<u>2A</u>) and friedelan-3β-ol(<u>2B</u>) were obtained.

Friedelin (<u>2A</u>)[4-7] : White needle, 68 mg, m.p. 258-260°C, Rf = 0.44 (silica gel/chloroform)
IR θ_{max} (KBr) 2900-2850, 1710 (C=0), 1460, 1380 cm^{-1};
MS, m/e (rel.intensity) 426 (M^+, 34), 411(9), 341(5), 302(20), 274(18), 273(34), 247(10), 218(28), 205(36), 191(21), 163(31), 69(100)
^1H NMR ($CDCl_3$) δ(ppm):0.72, 0.87, 0.92, 0.95, 1.01, 1.05, 1.18, (s, CH_3, 21H), 0.84 (d, CH_3, 3H), 1.2-1.62 (m, CH_2, CH, 23H), 2.37 (m, C<u>H</u>C=0,3H)
^{13}C NMR ($CDCl_3$) δ(ppm): 6.83 (C-23), 14.68 (C-24), 17.99 (C-25), 18.26 (C-7), 18.69 (C-26), 20.26 (C-27), 22.32 (C-1), 28.17 (C-20), 30.01 (C-17), 30.55 (C-12), 31.61 (C-30), 32.13 (C-28), 32.45 (C-15), 32.83 (C-21), 35.05 (C-29), 35.38 (C-19), 35.65 (C-11), 36.03 (C-16), 37.49 (C-9), 38.36 (C-13), 39.28 (C-22), 39.71 (C-14), 41.34 (C-6), 41.55 (C-2), 42.15 (C-5), 42.85 (C-18), 53.15 (C-8), 58.24 (C-4), 59.54 (C-10), 213.12 (C-3).

Anal.found : C, 84.68; H 12.04.Calcd.for $C_{30}H_{50}O$: C, 84.51; H 11.74

Friedelan-3 β-ol(<u>2B</u>)[6, 7] : White plates 115 mg, m.p. 278-280°C, Rf 0.34 (silica gel/chloroform). The color changed to pink upon treatment with Liebermann-Burchard reaction suggested it was triterpenoidal in nature.

IR θ_{max} (KBr) 3495 (−OH), 2950, 2850, 1450, 1385, 1020 cm^{-1}
MS, m/e (rel.intensity) 428 (M^+, 17), 413 (16), 304 (2), 275 (22), 249 (7), 218 (31), 205 (26), 165 (53), 95 (100)
^1H NMR ($CDCl_3$) δ(ppm):0.86, 0.97, 0.99, 1.17 (s, CH_3, 24H), 1.26-1.65 (m, CH_2, CH, 27H), 3.72 (s, C<u>H</u>-OH, 1H)

^{13}H NMR ($CDCl_3$) δ(ppm): 11.59 (C-23), 15.82 (C-24), 16.41 (C-1), 17.55 (C-7), 18.26 (C-25), 18.64 (C-26), 20.09 (C-27), 28.17 (C-20), 30.07 (C-17), 30.66 (C-12), 31.80 (C-30), 32.13 (C-28), 32.34 (C-15), 32.83 (C-21), 35.05 (C-29), 35.21 (C-2), 35.38 (C-11), 35.59 (C-19), 36.13 (C-16), 37.16 (C-9), 37.86 (C-5), 38.41 (C-13), 39.28 (C-22), 39.71 (C-14), 41.77 (C-6), 42.85 (C-18), 49.19 (C-4), 53.25 (C-8), 61.38 (C-10). 72.76 (C-3)

Anal.found : C, 83.96; H, 12.42. Calcd.for $C_{30}H_{52}O$: C, 84.11; H, 12.15

The third component (3) was a mixture of two compounds which could not be separated because of the identical Rf value. GC-MS showed the molecular ions of 414 and 412 corresponded to the molecular formulas $C_{29}H_{50}O$ and $C_{29}H_{48}O$ respectively. The color changed from pink to blue to green upon treatment with Liebermann-Burchard reaction suggested it was steroidal in nature.

Component (3) showed absorption band of -OH at 3500-3200 cm^{-1} so it was a steroidal alcohol. ^{1}H NMR spectrum showed signals of C$\underline{H}$-OH at δ3.52, -C$\underline{H}$=C$\underline{H}$ at δ5.09 and C=C$\underline{H}$ at δ5.35. The ^{1}H NMR spectrum agreed with the ^{13}C NMR spectrum, which showed the signals of C-OH at δ71.73 and C=C at δ121.68, 129.29, 138.31, 140.75.[8, 9, 10] ^{1}H NMR and ^{13}C NMR spectra of component 3 were identical with 1:1 mixture of β-sitosterol and stigmasterol.[11] TLC of component 3 has the same Rf value with that of the authentic sample. GC and co-injection GC of component 3 have the same retention times as that of β-sitosterol and stigmasterol and showed the composition of 35% β-sitosterol ($C_{29}H_{50}O$) and 65% of stigmasterol ($C_{29}H_{48}O$).

Rf 0.47 (silica gel/methanol : chloroform = 1 : 49)

IR θ_{max} (KBr) 3500-3200 (O-H), 3020 (C=CH), 2940, 2860, 1640, 1460, 1380, 1060, 1020, 970, 960, 800 cm^{-1}

MS, m/e (rel.intensity) 414 (M^+, 68), 396 (55), 381 (36), 329 (40), 273 (22), 255 (25), 213 (49), 43 (100); 412 (M^+, 63), 394 (14), 369 (14), 351 (21), 300 (30), 271 (35), 255 (51), 55 (100).

^{1}H NMR ($CDCl_3$) δ(ppm): 0.68-2.32 (m, C, CH, CH_2CH_3), 3.52 (b, OH), 5.09 (t, CH=CH) 5.35 (d, =CH-)

^{13}C NMR ($CDCl_3$) δ(ppm):

β-Sitosterol : 11.87 (C-18), 11.87 (C-29), 18.74 (C-26), 19.02 (C-21), 19.39 (C-19), 19.82 (C-27), 21.07 (C-11), 23.02 (C-28), 24.27 (C-15), 26.06 (C-25), 28.22 (C-16), 29.09 (C-23), 31.46 (C-2), 31.85 (C-7), 31.85 (C-8), 33.90 (C-22), 36.14 (C-20), 36.46 (C-10), 37.21 (C-1), 39.76 (C-12), 42.26 (C-4), 42.26 (C-13), 45.78 (C-24), 50.11 (C-9), 56.02 (C-17), 56.72 (C-14), 71.73 (C-3), 121.68 (C-6), 140.75 (C-5); Stigmasterol : 11.97 (C-29), 12.24 (C-18), 19.02 (C-26), 19.39 (C-19), 21.07 (C-11), 21.07 (C-21), 21.20 (C-27), 24.47 (C-15), 25.40 (C-28), 28.93 (C-16), 31.64 (C-2), 31.85 (C-7), 31.85 (C-8), 31.85 (C-25), 36.46 (C-10), 37.21 (C-1), 39.65 (C-12), 40.47 (C-20), 42.26 (C-4), 42.26 (C-13), 50.11 (C-9), 51.19 (C-24), 55.90 (C-17), 56.86 (C-14), 71.73 (C-3), 121.68 (C-6), 129.26 (C-23), 138.31 (C-22), 140.75 (C-5)

Anal.found : C, 84.14; H 12.08. Calcd.for $C_{29}H_{50}O$: C, 83.99; H 12.15
Calcd.for $C_{29}H_{48}O$: C, 84.40; H 11.72

The fourth component (4) was eluted with methanol : chloroform (1:19) as a white solid (680) mg) m.p.228-229°C (from ethyl acetate) Rf = 0.38 (silica gel/chloroform : methanol = 9:1). The IR spectrum of (4) showed absorption band of N-H at 3360 and 3250 cm^{-1}, aromatic nucleus at 1680, 1625, 1550 cm^{-1}, double bond at 3010-3080 cm^{-1}. ^{1}H NMR spectrum gave signals of aromatic protons at δ8.24 and 7.80, double bond at δ5.53, 5.02, signals of CH_3 at δ1.76, 1.90. These signals agreed with ^{13}C NMR (DEPT) signals of aromatic at δ144.82, 156.69, 141.35, 150.71, 153.18 signals of double bond at δ118.33, 121.21, signals of two methyl groups at 25.80 and 18.21

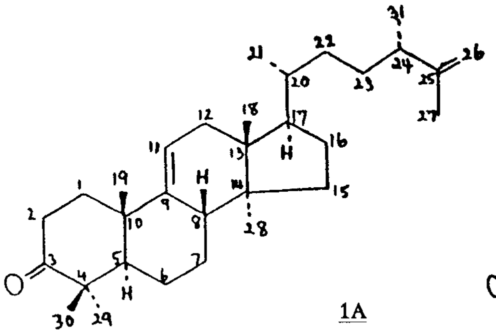
From all evidence component 4 was triacanthine or 6-amino-3-(γ, γ-dimethylallyl)-purine. To our knowledge this was the first report of the occurence of triacanthine in Euphorbiaceae. Triacanthine was isolated from *Gleditsia triacanthose*. It bears the same name as togholamine and chidlovine.[12] However, there was no ^{13}C NMR spectrum reported previously.

Triacanthine (4) : m.p. 228-299°C (lit. 228-229°C)[12]
UV λ_{max} (EtOH) 273 nm (ϵ12,500); λ_{max} (EtOH) (pH 1) 277 (18,300)
IR θ_{max} (KBr, cm^{-1}) 3360, 3250 (N-H), 1840, 1740, 1680, 1625, 1550 (aromatic)
MS, m/e (rel.intensity) 203 (M^+, 28), 188(62), 135(100), 108(52)

^{1}H NMR (acetone-d6, 250 MHz) δ(ppm) 1.76 (s, 3H, CH_3), 1.90 (s, 3H, CH_3), 5.02 (d, 2H, J=6.7 Hz, H-10), 5.53 (m, 1H, H-11), 7.80 (s, 1H, H-8), 8.24 (s, 1H, H-2)
^{13}C NMR (CD_3OD, 67.7 MHz) δ(ppm) 18.21 (C-13), 25.80 (C-14), 48.67 (C-10), 118.33 (C-11), 121.21 (C-12), 141.35 (C-5), 144.82 (C-2), 150.71 (C-6), 153.18 (C-8), 156.69 (C-4)

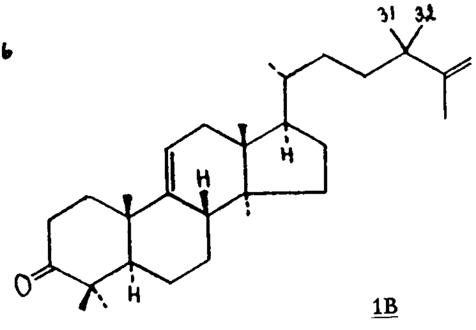
Anal.found : C, 59.21; H, 6.59; N, 34.20
Calcd.for $C_{10}H_{13}N_5$: C, 59.09; H, 6.45; N, 34.46

The fifth component (5), Rf = 0.24 (silicagel/methanol:chloroform = 1:9), was a mixture of two compounds which could not be separated because of the identical Rf value. It gave positive Liebermann-Burchard reaction, suggesting it was steroidal in nature. The IR spectrum of 5 exhibited a strong OH absorption and glycosidic band. The mass spectrum displayed similar fragmentation pattern as component 3, implying that this component was a glycoside derivative of β-sitosterol and stigmasterol. Hydrolysis of this glycoside gave aglycone which was similar to the mixture of β-sitosterol and stigmasterol in every respect such as GC, co-injection in GC, IR, TLC, m.p., mixed m.p., MS, ^{1}H NMR and ^{13}C NMR spectra



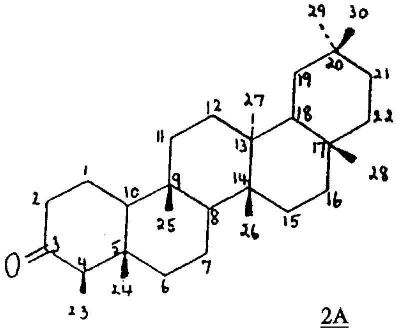
1A

$C_{31}H_{50}O$



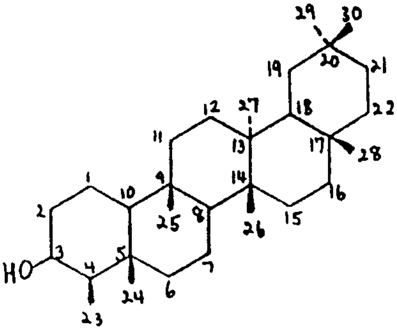
1B

$C_{32}H_{52}O$



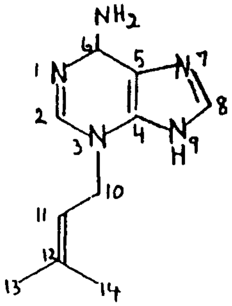
2A

Friedelin ($C_{30}H_{50}O$)



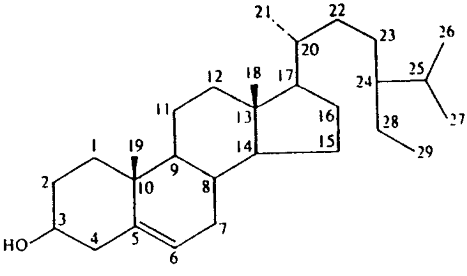
2B

Friedelan-3β-ol ($C_{30}H_{52}O$)



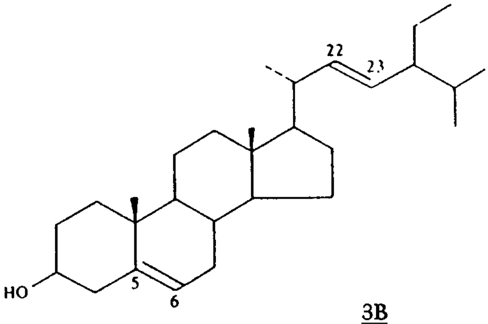
4

Triacanthine ($C_{10}H_{13}N_5$)



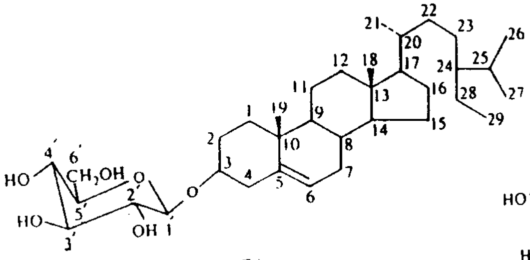
3A

β-Sitosterol ($C_{29}H_{50}O$)



3B

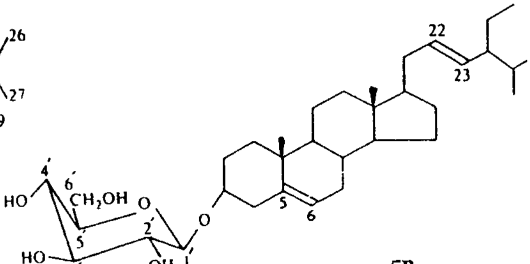
Stigmasterol ($C_{29}H_{48}O$)



5A

β-Sitosteryl-3-O-β-D-glucopyranoside

($C_{35}H_{60}O_6$)



5B

Stigmasteryl-3-O-β-D-glucopyranoside

($C_{35}H_{58}O_6$)

(see component 3). The sugar part gave the same Rf value as glucose in paper chromatography. From all data obtained for this component support the conclusion that it was a mixture of β-sitostery-3-O-β-D-glucopyranoside and stigmasteryl-3-O-β-D-glucopyranoside.

As *Bridelia tomentosa* Bl. has been used for medicinal purposes, some of the biological activities reported for some constituents to be present in the roots of this plant are described below. Friedelin (friedelan-3-one) has been reported for the treatment of cancerous cachexia[13] while friedelan-3β-ol has been shown to exhibit anti-inflammatory and anticonvulsant.[14] β-Sitosterol has been shown to be laxative,[15] anticholesteremics,[16] anti-inflammatory and antipyretic,[17] and antithrombotic.[18] Stigmasterol has been reported to be laxative,[15] and for hair treatment.[19] The β-glucoside has been shown to have growth promoting activity,[20] and to be active against P388 leukemia.[21] Triacanthine has been reported to have the action of sympatholytic, antispasmodic, vasodilating and somewhat sedative.[22]

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บทคัดย่อ

จากการทดลองหาองค์ประกอบทางเคมีของรากขนหนอนในผลสกัดคลอโรฟอร์ม สามารถแยกสารประกอบได้ 9 ชนิด ข้อมูลทางสเปกโทรเมตรีระบุว่าสารทั้งเก้าคือ 24-methyllanosta-9(11), 25-dien-3-one;24,24-dimethyllanosta-9(11), 25-dien-3-one;friedelin;friedelan-3-β-ol, β-sitosterol; stigmasterol; triacanthine, β-sitosteryl-3-O-β-D-glucopyranoside; stigmasteryl-3-O-β-D-glucopyranoside