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Constituents of Cissus quadrangularis Linn.(สารสำคัญของต้นเพ็ชรสังฆาต)

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ORIGINAL ARTICLE

Constituents of *Cissus quadrangularis* Linn.

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ABSTRACT

From the petroleum ether extract of *Cissus quadrangularis* Linn., three triterpenes and one phytosterol were isolated. The triterpenes were identified as lupenone, epifriedelinol, isoarborenol and the phytosterol as β-sitosterol. (Th. J. Pharm. Sci. 11 (4) 205-211 (1986))

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INTRODUCTION

The plant, *Cissus quadrangularis* Linn. (or "Phet-Sang-khaat", family Vitidaceae) is reputed to have some medicinal values. According to Pongboonrod (1), this plant has been used as carminative, antihemorrhoid, and used to promote fracture bone healing. In India this plant has also been known to have beneficial effect on fracture bone healing for many centuries and this effect had been proved on experimental animals (2, 3, 4). The studies on active constituents of this plant were done (5, 6) and the presence of 2 ketosteroids of unknown chemical structures were reported. Further study of this plant was done in 1983 (7), and the presence of two new tetracyclic triterpenes (3β, 21β dihydroxy-7-onocerene and 3β, 21 α-dihydroxy-7-onocerene) along with sitosterol, δ amyrin and δ amyron were reported. We report herein the isolation and structure determination of three pentacyclic triterpenes, along with β-sitosterol from *Cissus quadrangularis* Linn.

MATERIALS AND METHODS

Materials

Plant materials were collected from Nakornpathom province. The melting points (mp) of the compounds were determined on a melting point apparatus (Gallenkamp) uncorrected. Infrared (ir) spectra were determined on a Schimadzu model IR 440 spectrophotometer. All nuclear magnetic resonance (nmr) spectra were reported in ppm with tetramethylsilane as an internal standard. ^{1}H-nmr spectra were recorded on a Varian model FX 90 Q instrument and on a Varian A-60 D instrument. Mass spectra (ms) were obtained at low resolution on JEOL mass spectrometer model DX 300 at 70 ev. and high resolution on CEC-110 B mass spectrometer with electron impact ionization at 70 ev. All reported intensities were from the low resolution ms. Thin layer chromatography (tlc) was carried out on Silica gel G. [type 60 (E. Merck)] coated plated. Visualization of chromatograms were by Liebermann-Burchard reagent. Column chromatography (cc) was carried out on Silica gel - 60 (230-400 mesh, E. Merck). Solvent A was benzene : chloroform (3:1).

Extraction and chromatography

Fresh plant (50 kg) was blended with 95% ethanol in a Waring electric blender. It was then macerated twice for 3-day periods each with 95% ethanol (70 L and 30 L). The ethanolic extract was concentrated to a syrupy liquid (500 ml) and was diluted with distilled water (100 ml). Exhaustively extracted with petroleum ether gave on evaporation, 60 g residue. The 60 g residue was divided into six portions (10 g each), each portion was subjected to column chromatography in the same manner. Column chromatography of 10 g sample on silica gel (300 g) in a flat bottom column (100 mm i.d.) employing solvent A gave compound 1, 2, 3 and 4. The elution pattern of the column was shown in table 1.

TABLE 1. Elution pattern of silica gel column chromatography

Fraction (25 ml)	Eluent	Compound
1 - 12	Benzene : Chloroform (3:1)	—
13 - 24	"	1
25 - 46	"	2
47 - 77	"	3
78 - 90	"	(3 + 4)
91 - 115	"	4
116	Methanol	—

Compound 1 (112 mg), appeared as white crystalline needles from n-hexane, mp 165-166°C, exhibited the following properties : ir ν_{max} KBr (cm^{-1}) : 3090, 2950, 2875, 1720, 1655, 1460, 1380, 890, 875; ^{1}H-nmr (90 MHz, $CDCl_3$) : δ 0.80 (3H, s), 0.94 (3H, s), 0.90 (3H, s), 1.03 (3H, s), 1.07 (6H, s),

* Separated by silica gel column chromatography using solvent A as an eluent.

1.68 (3H, s), 4.64 (2H, d); ms *m/z* (% rel int.) : 424 (M^+, 33) 409 (11), 381 (9), 314 (13), 245 (16), 218 (25), 205 (100), 203 (29), 189 (36), 109 (88), 99 (100).

DNP derivative of compound 1

Two milligrams of compound 1 was dissolved in cold methanol (5 ml). The slightly acidic methanolic solution (5 ml of dil HCl + 10 ml of methanol) of DNP was added. The solution mixture was concentrated on a steam bath to yield a yellow solid. It was then recrystallized in chloroform/methanol to yield crystalline needles, mp 205-207° C.

Compound 2 (120 mg), appeared as white crystals from petroleum ether, mp 272-275°C, exhibited the following properties : ir ν_{max} KBr (cm^{-1}) : 3620, 3480, 2920, 2870, 1450, 1380, 995, 975, 915 ; ^{1}H-nmr (60 MHz, $CDCl_3$) : δ 0.69 (3H, s), 0.86 (3H, s), 1.00 (3H, s), 1.14 (3H, s), 1.35 (3H, s), 1.42 (3H, s), 1.50 (3H, s), 1.52 (3H, s), 3.72 (1H, m); ms *m/z* (% rel. int.) : 429 (M^+, 33), 414 (34), 411 (100), 396 (63), 219 (39), 192 (29), 150 (25), 124 (48), 109 (71), 107 (47), 95 (100).

Compound 3 (150 mg), appeared as white crystals from n-hexane, mp 275-278°C, exhibited the following properties : ir ν_{max} KBr (cm^{-1}) : 3475, 2950, 2875, 1630, 1475, 1450, 1385, 1375, 1365, 1060, 1030, 980 ; ^{1}H-nmr (60 MHz, $CDCl_3$) : δ 0.60 (3H, s), 0.68 (3H, s), 0.74 (3H, s), 0.80 (3H, s), 0.96 (3H, s), 0.99 (3H, s), 1.10 (6H, s), 1.27 - 1.87 (2H, m), 3.20 (1H, m), 5.21 (1H, m) ; ms *m/z* (% rel. int.) : 426 (M^+, 75), 411 (100), 408 (31), 394 (27), 393 (81), 259 (79), 241 (44), 137 (47), 133 (39), 123 (40), 121 (48), 119 (52), 109 (65), 107 (52), 95 (95).

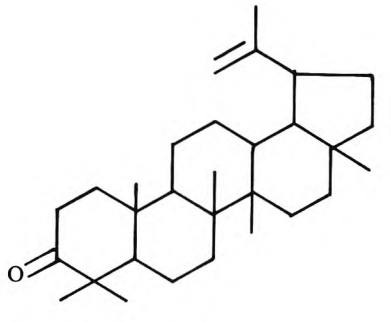
Acetate derivative of compound 3

Five milligrams of compound 3 was heated in a steam bath for 2 hours with 5 ml acetic anhydride and 5 ml pyridine. The solution was concentrated under reduced pressure. The residue was crystallized in n-hexane to yield crystalline needles, mp 285-288°C.

Compound 4 (3.729 g) appeared as white crystalline needles from absolute alcohol, mp 139°C, exhibited the following properties: ir ν_{max} KBr (cm^{-1}) : 3450, 2920, 2860, 1649, 1500, 1370, 1004: ^{1}H-nmr (90 MHz, $CDCl_3$) : δ 0.68 (3H, s), 1.01 (3H, s), 3.49 (1H, broad s), 5.51 (1H, d).

RESULTS

Compounds 1, 2, 3, and 4 were identified as lepenone, epifriedelinol, isoarborenol and β-sitosterol respectively.



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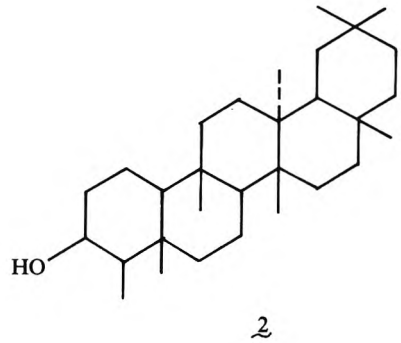

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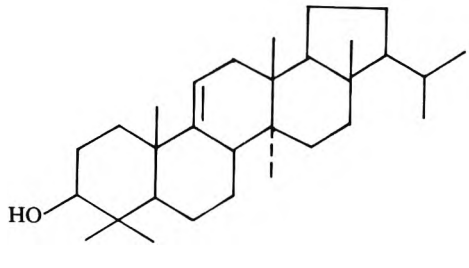

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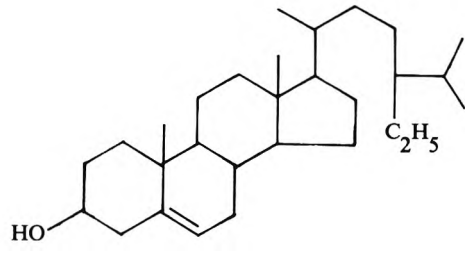

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DISCUSSION and CONCLUSION

Column chromatography of crude petroleum ether extract of *Cissus quadrangularis* Linn. yielded four compounds, of which three are triterpenes and the other one is a sterol.

Compound 1 has been identified as a known triterpene called lupenone. The mass spectral fragmentation of compound 1 (see figure 1) indicated that it is a pentacyclic triterpene of lupane type, showing melecular ion at *m/z* 424 and the fragments at *m/z* 218, 205 and 186. The ir spectrum showed a ketone functional group at 1720 cm^{-1} and a terminal alkene group at 1645 cm^{-1}. The ^{1}H-nmr spectrum showed the presence of six unsplit methyl groups, one methyl group broaded by allylic couplings and a terminal methylene group. The melting point and the spectral data of compound 1 were in full agreement with those of literature values (8). Moreover, the melting point of DNP derivative of compound 1 showed identical value with that of DNP derivative of lupenone in the literature (9).

Compound 2 has been identified as a known triterpene called epifriedelinol by comparison of its melting point and ir spectrum with those of published values (10). The computer search of mass spectrum collection at Massachusetts Institute of technology (MIT), indicated that the spectrum of epifriedelinol is the most probable spectrum for compound 2. Moreover the ^{1}H-nmr spectrum of compound 2 showed the series of peaks corresponding to the peaks that one might expect for epifriedelinol.

Compound 3 has been identified as a known triterpene called isoarborenol by comparison of its spectral properties with the reported ^{1}H-nmr and mass spectrum of isoarborenol (11). Moreover, the melting point of compound 3 and its acetate derivative were in full agreement with those of isoarborenol and its acetate, reported in the literature (12).

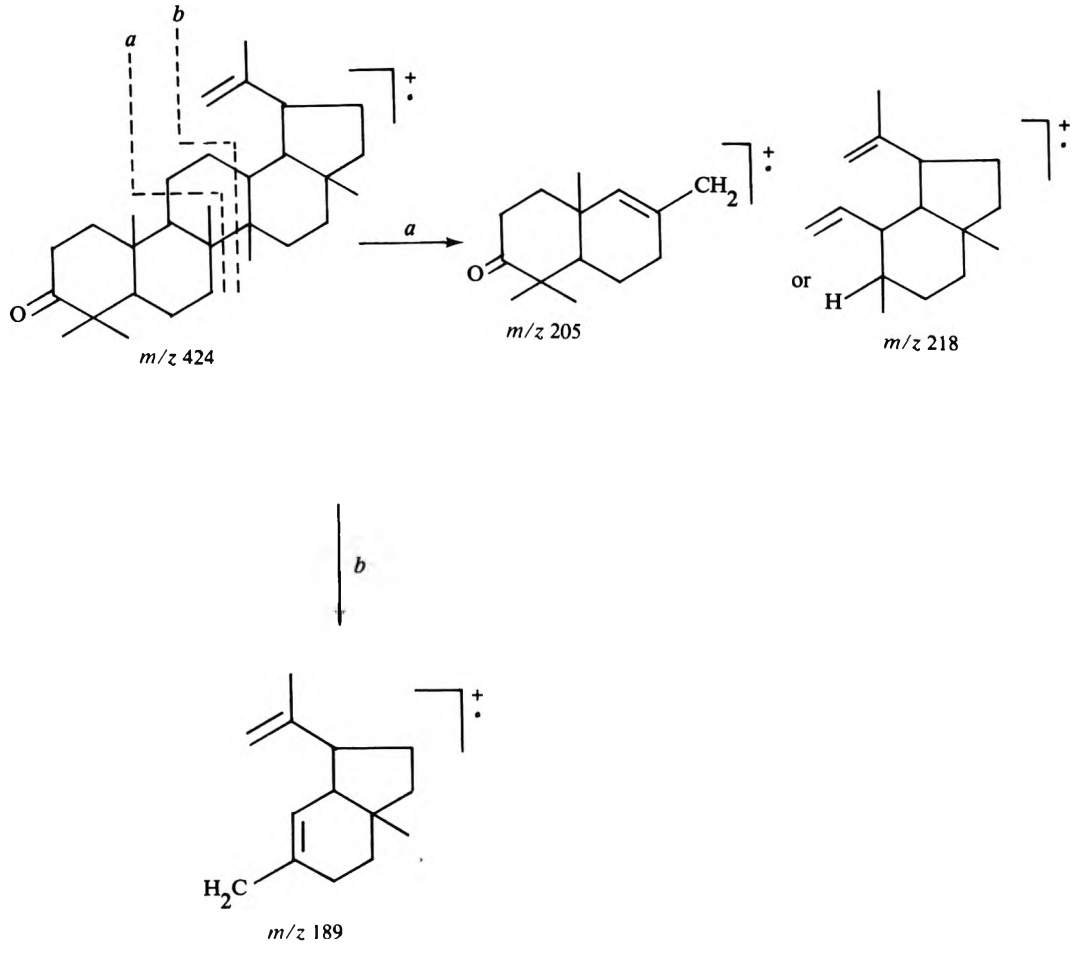

FIGURE 1. Lupenone (1), ms fragmentation pattern

Compound 4 which is apparently the main constituent has been identified as a common phytosterol called β-sitosterol by comparison of its melting point with that of the literature value (13). Futher proof of the identity of compound 4 was obtained by a peak by peak comparison of the ir and ^{1}H-nmr spectra of our sample with those of published spectra (14, 15).

So far no pharmacological study of compounds isolated in these investigation are reported. All isolated compounds are simple triterpenes and sterol which are found to be widely distributed in various plants.

The data obtained from this investigation together with the report of Bhutani *et al.* (7) are not sufficient to prove Sen's proposal (5, 6) about the presence of bone healing principle, ketosteroid, until more exhaustive studies of non-polar fraction of *Cissus quadrangularis* Linn. are done.

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สารสำคัญของต้นเพ็ชรสังฆาต

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บทคัดย่อ

จากสิ่งสกัดด้วย Petroleum ether ของต้นเพ็ชรสังฆาต สามารถแยกและพิสูจน์เอกลักษณ์ ได้สารจำพวก Triterpene 3 ชนิด คือ lupenone, epifriedelinol, isoarborenol และสารจำพวก phytosterol 1 ชนิด คือ β-sitosterol (ไทยเภสัชสาร 11 (4) 205-211 (2529))

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