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# Chemical Constituents of Petroleum Ether Extract from *Nervilia foadii*

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**Abstract:** Five compounds were isolated from the leaves of petroleum ether extract of *Nervilia foadii* by silica gel column and recrystallization. These compounds were identified through spectral analysis (IR, UV, $^{1}$HNMR, $^{13}$CNMR, MS) as lupane-17-(29-propylol) (1), octacosanoic acid (2), 23-lanostene-3-alcohol (3), 22, 24-cyc-loartenone-3-α-p-hydroxy-cassia-acid (4), hexodecanoic acid (5).

**Key words:** *Nervilia foadii*, chemical constituents, spectral analysis

## INTRODUCTION

*Nervilia foadii* is well-known traditional Chinese medicinal herbs and extensively used to protect liver and treat hepatitis in countryside (Ling, 2001; Du *et al*., 2005a, b). There are a large number of Nervilia foadii in Guangxi Zhuang Autonomous. *Nervilia foadii* is found only in China (Du *et al*., 2005b). Essential oil is the major constituent in *Nervilia foadii* (Du *et al*., 2005b). But there are few reports about the chemical constituents in *Nervilia foadii*. Petroleum ether extract from *Nervilia foadii* inhibited the proliferation of 2.2.15 cells obviously. In the present study, the chemical constituents of petroleum ether fraction of the ethanolic extract of *Nervilia foadii* were investigated.

## MATERIALS AND METHODS

**Plant material:** The whole plant collected in Shangling City of Guangxi Province was identified by Zijing Zhou, a Botany Professor of Biology Department of Guangxi Chinese Medical University, where a Voucher specimen has also been deposited. The whole plant, after washing with water and air-drying for several days, were powdered.

**General:** IR spectra were carried out on a Bruker Vector 22 spectrophotometer in KBr pellets. UV spectra were recorded with a Shimadzu UV-2501PC spectrophotometer. $^{1}$H, $^{13}$C and 2D NMR spectra (including $^{1}$H-$^{1}$H COSY, HSQC and HMBC) were recorded in $CD_3OD$ and DMSO-$d_6$ on a Bruker DRX-400 instrument operating at 400 MHz for $^{1}$H and 100 MHz for $^{13}$C. Chemical shifts are reported in ppm (δ). Fast atom bombardment (FAB) mass spectra were recorded in the positive and negative ion mode on a VG 70 SEQ instrument using glycerol as the liquid matrix. TLC was carried out on precoated silica gel 60 F 254 plates (Merck).

**Extraction and isolation:** Dried and powdered plant material (7.82 kg, whole plant) of *Nervilia foadii* (Hence) Schltr was extracted exhaustively with 95% ethanol. The filtrate was concentrated under reduced pressure and then extracted with petroleum ether. The petroleum ether extract was evaporated under reduced pressure and subjected to CC on silica gel (Qingdao, 100-200 mesh) eluted with a petroleum ether: EtOAc gradient and recrystallization yielding compound 1 (431 mg), 2 (76 mg), 3 (187 mg), 4 (56 mg), 5 (315 mg).

## RESULTS AND DISCUSSION

**Spectral analysis:** Compound 1 was obtained as a white crystal. The UV spectra showed the max absorption bands at 204 nm. In the IR spectral analysis, the peak at 3289 $cm^{-1}$, a broad band, means O-H stretching. The peaks at 2933 and 2870 $cm^{-1}$ showed the C-H bands stretching of saturated hydrocarbons (CH, $CH_2$ and $CH_3$). The IR spectra also showed C = C absorption at 1638 and 1496 $cm^{-1}$. The result of the FAB Mass spectra showed a molecular ion 454 $[M]^+$. In the EI mass, 439 (M-$CH_3$), 436, 411 and 395 (M-$C_3H_7O$) were given. The $^{13}$C NMR spectra also showed two carbons absorptions of one C = C; one at 147.45 δ due to binding with three carbons and another at 111.86 δ. Total thirty-two carbons were found in the $^{13}$C NMR. The peak assignments of compound 1 in the $^{13}$C-NMR spectra are summarized in Table 1. The chemical shift assignment of the $^{1}$HNMR

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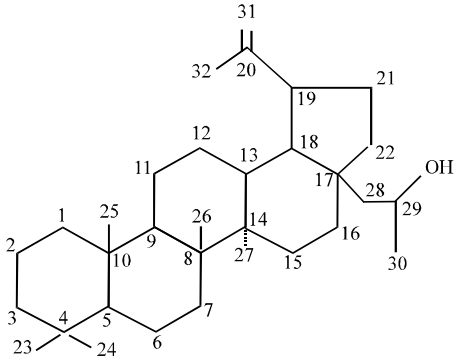


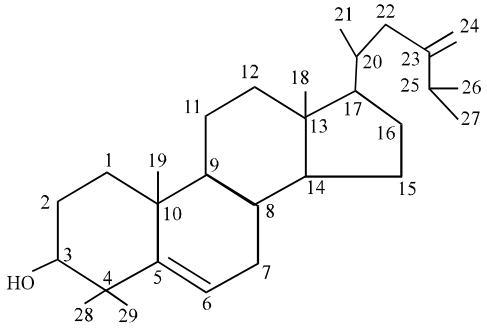
Compound 1

Table 1: Chemical shifts (ppm) for the carbons of compound 1

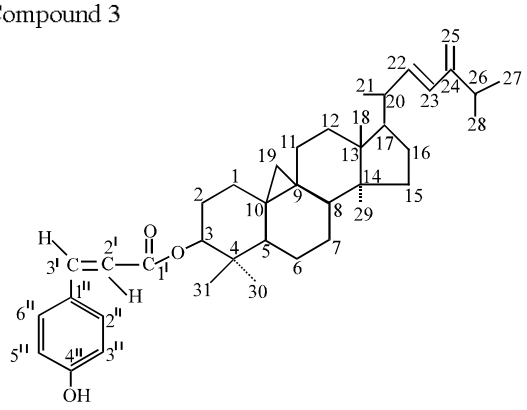
| No. | ppm (δ) | No. | ppm (δ) | No. | ppm (δ) |
|---|---|---|---|---|---|
| 1 | 34.545 | 12 | 35.390 | 23 | 18.364 |
| 2 | 24.696 | 13 | 44.660 | 24 | 28.841 |
| 3 | 24.100 | 14 | 48.940 | 25 | 18.731 |
| 4 | 45.332 | 15 | 33.973 | 26 | 17.755 |
| 5 | 46.859 | 16 | 27.044 | 27 | 19.509 |
| 6 | 25.182 | 17 | 52.396 | 28 | 27.227 |
| 7 | 30.833 | 18 | 43.390 | 29 | 55.568 |
| 8 | 49.344 | 19 | 52.210 | 30 | 20.470 |
| 9 | 30.314 | 20 | 147.435 | 31 | 111.859 |
| 10 | 39.499 | 21 | 28.152 | 32 | 21.470 |
| 11 | 26.812 | 22 | 34.875 | | |

spectral data supported the identity of the compound 1 as a triterpenoid. In the $^{1}$HNMR, there were many peaks in δ 0.7-2.2, indicating that the structure of triterpenoid. δ 4.74 (1H, S) and δ 4.61(1H, S) were adsorption of two protons of C = C. From the foregoing therefore, compound 1 was confirmed as lupane-17-(29-propylol).

Compound 3 was obtained as a white crystal (petroleum ether). The UV spectra showed the max absorption bands at 204 nm. In the IR spectral analysis, the peak at 3424 cm$^{-1}$, a broad band, means O-H stretching. The peaks at 2935 and 2866 cm$^{-1}$ showed the C-H bands stretching of saturated hydrocarbons ($CH_2$ and $CH_3$). The IR spectra also showed C = C absorption at 1624 cm$^{-1}$. The result of the FAB Mass spectra showed a molecular ion 412 $[M]^+$. In the EI mass, 394 (M-$H_2O$) was given. The $^{13}$C NMR spectra also showed four carbons absorptions of two C = C; two at 140.81 δ and 138.28δ due to binding with three carbons. Another two were at 121.69 (= CH) and 110.10 (= $CH_2$). Total twenty-nine carbons were found in the $^{13}$C NMR. The peak assignments of compound 3 in the $^{13}$C-NMR spectra are summarized in Table 2. The chemical shift assignment of the $^{1}$HNMR spectral data supported the identity of the compound 3 as a triterpenoid. In the $^{1}$HNMR, there were many peaks in δ 0.7-2.288, indicating that the structure of triterpenoid. δ 4.74 (1H, S) and δ 4.61(1H, S) were adsorption of two end protons of C-24. δ 5.35 (1H, S) was proton of C-6. From the foregoing therefore, compound 3 was confirmed as 23-lanostene-3-alcohol.



Compound 3



Compound 4

Table 2: Chemical shifts (ppm) for the carbons of compound 3

| No. | ppm (δ) | No. | ppm (δ) |
|---|---|---|---|
| 1 | 40.263 | 16 | 25.385 |
| 2 | 31.305 | 17 | 56.912 |
| 3 | 71.826 | 18 | 18.994 |
| 4 | 40.433 | 19 | 21.047 |
| 5 | 140.808 | 20 | 50.233 |
| 6 | 121.686 | 21 | 20.793 |
| 7 | 31.887 | 22 | 42.367 |
| 8 | 31.941 | 23 | 138.281 |
| 9 | 55.987 | 24 | 110.778 |
| 10 | 36.552 | 25 | 51.256 |
| 11 | 24.377 | 26 | 12.210 |
| 12 | 37.305 | 27 | 12.057 |
| 13 | 39.727 | 28 | 19.392 |
| 14 | 58.578 | 29 | 21.217 |
| 15 | 29.378 | | |

Compound 4 was obtained as a white crystal (petroleum ether). The UV spectra showed the max absorption bands at 204, 258 and 309 nm. 258 and 309 nm was adsorption of π-π* and n-π. In the IR spectral analysis, the peak at 3368 cm$^{-1}$, a broad band, means O-H stretching. The peaks at 2934 cm$^{-1}$ and 2870 cm$^{-1}$ showed the C-H bands stretching of saturated hydrocarbons 0. The IR spectra also showed benzene absorption at 1626, 1604 and 1515 cm$^{-1}$. 1201 and 1168 cm$^{-1}$ showed the C-O-C stretching. The result of the FAB Mass spectra showed a molecular ion 584 $[M]^+$. In

Table 3: Chemical shifts (ppm) for the carbons of compound 4

| No. | ppm (δ) | No. | ppm (δ) |
|---|---|---|---|
| 1 | 32.841 | 21 | 18.348 |
| 2 | 27.177 | 22 | 116.370 |
| 3 | 78.861 | 23 | 129.884 |
| 4 | 32.446 | 24 | 147.432 |
| 5 | 46.907 | 25 | 111.846 |
| 6 | 24.695 | 26 | 30.308 |
| 7 | 30.542 | 27 | 21.497 |
| 8 | 43.528 | 28 | 20.836 |
| 9 | 29.411 | 29 | 19.148 |
| 10 | 30.377 | 30 | 14.467 |
| 11 | 25.067 | 31 | 23.721 |
| 12 | 36.377 | 1′ | 116.606 |
| 13 | 45.317 | 2′ | 115.890 |
| 14 | 48.909 | 3′ | 143.193 |
| 15 | 35.380 | 1″ | 127.518 |
| 16 | 27.024 | 2″ | 132.341 |
| 17 | 52.168 | 3″ | 115.043 |
| 18 | 17.764 | 4″ | 156.739 |
| 19 | 30.970 | 5″ | 115.043 |
| 20 | 41.507 | 6″ | 132.341 |

the EI mass, 437 (M-147), 422 (M-147-$CH_3$), 421 (M-147-O), 394 (M-147-$C_3H_7$), 356 (M-147-$C_5H_{11}$), 312 (M-147-$C_9H_{17}$), 283 (M-147-$C_9H_{17}$-CHO), 147 (M-437) and 119 (M-437-CO) were given. The $^{13}C$ NMR spectra also showed 6 carbons absorptions of three C = C; two at 140.81 and 138.28δ due to binding with three carbons. Total 29 carbons were found in the $^{13}C$ NMR. The peak assignments of compound 4 in the $^{13}C$-NMR spectra are summarized in Table 3. The chemical shift assignment of the $^{1}$HNMR spectral data supported the identity of the compound 4 as a triterpenoid. In the $^{1}$HNMR, δ 7.63 (1H d J = 16), δ 6.82 (1H d J = 16), δ 6.85 (1H d J = 13) and δ 5.85 (1H d J = 13) were adsorptions of four protons of benzene. From the foregoing therefore, compound 4 was confirmed as 22,24-cyc-loartenone-3- α-p-coumaric acid ester.

Compounds 2 and 5 were identified as Octacosanoic acid and Hexodecanoic acid, respectively.

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