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TRITERPENES FROM THE ROOTS OF *CODONOPSIS PILOSULA*

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SUMMARY

From the roots of Codonopsis pilosula (Franch) Nannf (Campanulaceae) five triterpenoids: taraxerol, taraxeryl acetate, 14-α-taraxeran-3-one and D:B-friedoolean-5-ene-3-β-ol as well as α-spinasterone were isolated. Their structures have been identified by MS, ^{1}H-, ^{13}C-NMR spectroscopy and comparison with reported data.

I - INTRODUCTION

Codonopsis pilosula (Franch) Nannf is one of the most famous traditional medicine and sometimes as cheaper substitute like ginseng in Chinese and Vietnamese drugs. It has been used for a long time and still being used widely today as a remedy for appetite, psychoneurosis, fatigue, dyspepsia and possessing adaptogenic, anti-stress properties [1]. Our previous study on the roots of *C. pilosula* (Franch) Nannf has led to the isolation and structural identification of lobetyol, 5-hydroxymethyl-2-furandehyde as well as bis-(2-ethylhexyl)-phthalate [2]. In this paper we report the isolation and structural elucidation of four triterpenoids, taraxerol (**1**), taraxeryl acetate (**2**), 14-α-taraxeran-3-one (3-taraxeranone, **3**), D:B-friedoolean-5-en-3-α-ol (**4**) and α-spinasterone (**5**).

II - EXPERIMENT

1. General

Optical rotation $[\alpha]_D$: Digital Polarimeter Jasco DIP 1000. EI-MS: ADM 402, 70 eV, Finnigan TSQ 700. NMR: VARIAN 300 spectrometer at 300 MHz (^{1}H) and 75.5 MHz (^{13}C, ^{13}C-APT). Chemical shifts were referenced to internal TMS (δ = 0, ^{1}H) and $CDCl_3$ (δ = 77.0, ^{13}C). CC: Silica gel 60, 0.06 - 0.20 mm (Merck) for the first column, silica gel 60, 40-63 μm (Merck) for the following columns. TLC: Silica gel 60 F-254 (Merck).

2. Plant material

The roots of *C. pilosula* were bought in Hanoi market, Vietnam in May 2005. The species was identified by Dr. Ngo Van Trai, Institute of Materia Medica, Hanoi. A voucher specimen (Nr. 1) is deposited in the Institute of Chemistry, VAST, Hanoi.

3. Extraction and isolation

The ground and dried roots of *C. pilosula* (2.4 kg) were extracted four time with MeOH (95%) at room temperature. MeOH was evaporated *in vacuo*, and the aq. solution (1.25 kg) was partitioned with *n*-hexane followed by EtOAc and n-BuOH (each four time), giving 45.0 g, 21.0 g and 29.3 g extracts, respectively. The n-hexane extract was separated on silica gel using n-hexane-$CHCl_3$ (20:80→90:10) and then $CHCl_3$-MeOH (98:2 → 90:10) to afford 24 fractions (F1-F24, 150 ml/Fr.). Compounds **1**-**5** were further purified by CC on silica gel or

crystallization.

Table 1: ^{1}H-NMR data of taraxeryl acetate (**2**) and α-spinasterone (**5**) (300 MHz, δppm, $CDCl_3$, J in Hz)

H	**2**	**5**	**5** [8]
1	1.39 *m* 1.92 *dd* (14.6; 3.1)	1.46 *m*; 2.13 *ddd* (6.1; 14.6; 14.6)	1.47 *m*; 2.12 *ddd* (6.1; 14.6; 14.6)
2	1.66 *m*; 2.02 *m*	2.28 br *d* (14.5)	2.28 br *d* (15)
3	4.45 *dd* (10.3; 6.2)	-	-
4	-	2.24 *m*	2.23 *m*
5	1.33 *m*	1.80 *m*	1.81 *m*
6	1.43 *m*, 1.46 *m*	1.83 *m*	1.82 *m*
7	1.29 *m*,1.62 *m*	5.18 *m*	5.18 *m*
9	1.54 *m*	1.77 *m*	1.76 *m*
11	1.42-1.46 *m*	1.56, 1.75 *m*	1.55, 1.75 *m*
12	0.93 - 1.33 *m*	1.27, 2.04	1.27, 2.04 *m*
14	-	1.83 *m*	1.83 m
15	5.53 *dd* (7.0; 3.2)	1.39 *m*, 1.50 *m*	1.40 *m*, 1.52 *m*
16	1.65 *m*, 2.01 *m*	1.29 *m*, 1.77 *m*	1.29 *m*, 1.67 *m*
17	-	1.30 *m*	1.30 *m*
18	1.03 *m*	0.58 *s*	0.58 s
19	1.43 *m*, 2.05 *m*	1.02 *s*	1.02 *s*
20	-	2.04 *m*	2.05 *m*
21	1.32 - 1.42 *m*	1.03 *d* (6.8)	1.03 *d* (6.7)
22	1.32 - 1.42 *m*	5.13 *dd* (8.4; 15.0)	5.16 *dd* (8.5; 15.2)
23	0.95 *s*	5.02 *dd* (8.5; 15.0)	5.02 *dd* (8.8; 15.3)
24	0.82 *s*	1.55 *m*	1.56 *m*
25	0.91 *s*	1.56 *m*	1.57 *m*
26	1.09 *s*	0.82 *d* (6.2)	0.82 *d* (6.1)
27	0.88 *s*	0.84 *d* (6.4)	0.84 *d* (6.7)
28	0.86 *s*	1.19 *m*; 1.41 *m*	1.18 *m*; 1.41 *m*
29	0.95 *s*	0.81 *t* (6.4)	0.81 *t* (7.3)
30	0.91 s	-	-
$CO\underline{CH_3}$	2.04 *s*	-	-

Table 2: ^{13}C-NMR data of compounds **1-5** ($CDCl_3$, 75.5 MHz, δppm)

C	**1***	**2**	**3**	**4**	**5**
1	38.56	37.73	38.31	18.30	38.79
2	26.60	28.86	28.22	27.88	38.15
3	78.65	80.98	212.96	76.32	211.68
4	38.83	39.01	42.16	40.86	44.26
5	55.39	55.63	53.08	141.45	42.88
6	18.68	18.76	18.30	121.96	30.10
7	35.63	35.15	35.36	23.71	116.88
8	38.55	37.92	39.28	47.45	139.33
9	48.56	48.76	42.79	34.88	48.85
10	37.81	37.72	37.45	49.70	34.44
11	17.39	17.59	18.30	34.65	21.76
12	36.51	35.83	35.65	30.41	39.35
13	37.54	37.41	36.03	39.33	43.37
14	157.76	157.78	58.19	37.87	55.02
15	116.56	116.84	30.03	32.12	23.02
16	37.40	36.70	32.79	36.07	28.55
17	37.62	37.58	39.70	30.15	55.85
18	49.68	49.20	59.44	43.08	11.98
19	41.17	41.23	41.55	35.12	12.34
20	28.64	28.86	28.22	28.32	40.86
21	34.95	33.72	32.44	33.16	21.76
22	33.55	33.13	30.54	38.99	137.92
23	27.75	28.04	18.74	29.02	129.37
24	15.33	15.59	6.93	25.53	51.25
25	15.33	16.67	14.73	16.30	31.91
26	29.63	29.99	18.74	19.70	19.06
27	25.76	25.99	32.13	18.52	21.45
28	29.73	29.89	31.83	32.10	25.45
29	33.13	33.41	35.07	34.59	12.34
30	21.15	21.42	20.33	32.47	-
C=O	-	170.82	-	-	-
$COCH_3$	-	21.36	-	-	-

* $CDCl_3$:CD_3OD (95:5)

*a) Taraxerol (**1**)*

Fractions 12-13 (Fr.12-14, 1.35g) were separated on silica gel, eluted with hexane-EtOAc (90:10) and then crystallization to give 360 mg (0.0150 %). $[\alpha]^{24}_{D}$ -22^{O} (*c* 1.0, $CHCl_3$). EI-MS 70 eV, *m/z* (rel. int.): 426 $[M]^+$ (16), 411

$[M-15]^+$ (14), 302 (52), 287 (40), 218 (37), 205 (38), 204 (100), 189 (30), 135 (19), 121 (24), 107 (22), 95 (23);). ^{1}H-NMR ($CDCl_3$, 300 MHz, δ ppm): 0.97 (Me-23), 0.82 (Me-24), 0.95 (Me-25), 1.09 (Me-26), 0.91 (Me-27), 0.80 (Me-28), 0.93 (Me-29), 0.91 (Me-30). ^{13}C-NMR (125 MHz, $CDCl_3$+CD_3OD): data see table 2.

*b) Taraxeryl acetate (**2**)*

Compound **2** was isolated from Fr. 4+5 by CC (silica gel, *n*-hexane-EtOAC 98:2). White needles from EtOAc, yield 60 mg (0.0032 %). ESI-MS (*m/z*): 491 $[M+Na]^+$ ($C_{32}H_{52}O_2$). EI-MS, 70 eV, *m/z* (rel. int.): 468 $[M]^+$ (41), 453 (24), 408 (20), 344 (60), 329 (26), 269 (24), 218 (40), 204 (100), 189 (30), 135 (18), 121 (17), 109 (21) 107 (10), 95 (24). ^{1}H- and ^{13}C-NMR data see Table 1 and 2.

*c) 3-Taraxeranone (14-α-taraxeran-3-one, **3**)*

Compound **3** was isolated from Fr.10 by CC (silica gel, *n*-hexane-EtOAC-$CHCl_3$, 90:10:1). White needles from EtOAc, yield 56mg (0.0023 %). $[\alpha]^{24}_D$ — 45 (*c* 2, $CHCl_3$, lit. [5] + 31^O). ESI-MS, *m/z*: 449 $[M+Na]^+$ ($C_{30}H_{50}O$). EI-MS, 70 eV, *m/z* (rel. int.): 426 $[M]^+$ (87), 411 (29), 341 (14), 302 (32), 273 (63), 205 (54), 191 (17), 179 (46), 163 (51), 123 (100), 109 (77), 95 (82), 69 (47); ^{1}H-NMR ($CDCl_3$, 300 MHz, δ ppm): 1.05 (Me-23), 1.00 (9H, Me-24, Me-25, Me-29), 1.18 (Me-26), 0.87 (Me-27), 0.88 (Me-28), 0.95 (Me-30). ^{13}C-NMR data see table 2.

*d) D:B-friedoolean-5-ene-3-β-ol (**4**)*

Compound **4** was isolated from Fr.10 and purified by CC [silica gel, n-hexane-EtOAc-$CHCl_3$ (90:10:1)]. Powder from EtOAc, yield 28 mg (0.0012 %). EI-MS, 70 eV, *m/z* (rel. int.): 426 $[M]^+$ (18), 409 (30), 274 ($C_{20}H_{34}$, 100), 259 (92), 205 ($C_{14}H_{21}O$, 47), 137 ($C_{10}H_{17}$, 30), 134 (45), 109 (46), 95 (42), 81 (23); ^{1}H-, ^{13}C-NMR data see Table 1 and 2.

*e) α-Spinasterone [(22E)-5α-stigmasta-7,22-diene-3-one (**5**)]*

Compound **5** was isolated from Fr.10 and purified by CC [silica gel, n-hexane-EtOAC-$CHCl_3$ (90:10:5)]. White needles from EtOAc, yield 45 mg (0.0019 %). EI-MS 70 eV, *m/z* (rel. int.): 410 $[M]^+$ (56), 397 (32), 395 $[M-Me]^+$, 367 $[M-C_3H_7]^+$ (52), 298 $[M-C_8H_{16}]^+$ (32), 271, 269 (loose of the side chain, 75), 257 (13), 244 (38), 229 (77), 95 (40), 83 (22), 55 (24). ^{13}C-NMR data see table 2.

III - RESULTS AND DISCUSSION

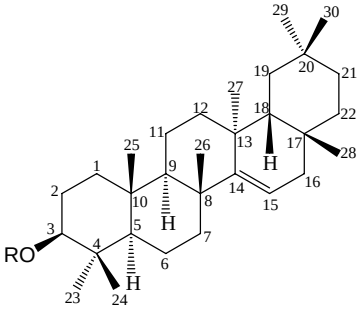
The residue of an ethanol extract of *C. pilosula* was partitioned with n-hexane, EtOAc, *n*-BuOH, successively. The n-hexane extract, after evaporation of solvent was chromatographed on column over silica gel and then crystallization to afford compounds **1**-**5**. Compounds **1**-**5** showed no fluorescence under UV light with λ_{max} 254 and 366 nm.

The EI-MS spectrum of **1** gave a mol peak at *m/z* 426 $[M]^+$, corresponding to the molecular formula $C_{30}H_{50}O$. The APT and ^{13}C-NMR spectra showed the presence of 30 carbons (CH_3x8, CH_2x10, CHx5, Cqx7), suggested that **1** has a triterpene skeleton. This was further confirmed by the signals of 8 tertiary methyl signals in the ^{1}H-NMR spectrum. One double bond was confirmed by olephinic methine signal at δ_H 5.53 (*dd*, *J* = 7.0; 3.2 Hz) and δ_C 157.76 (C-14), 116.56 (C-15). The structure of **1** was identified as taraxerol (14-taraxeren-3β-ol) by comparison of its ^{1}H- and ^{13}C-NMR spectra (Table 1) with reported data [3, 4]. Taraxerol showed antiulcer activity and is a cancer chemopreventive agent. It was isolated for the first time from *Taraxacum officinale* and then frequently found in other plants (*Rhododendron* spec., *Euphorbia* spec.) [3, 4].

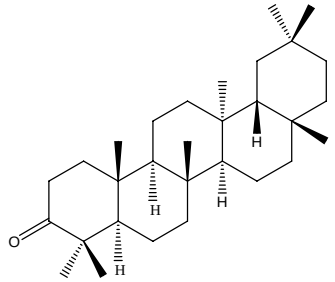
Compound **2** was obtained as white needles from *n*-hexane extract using column chromatography on silica gel. The EI-MS spectrum gave a mol peak at *m/z* 468 $[M]^+$ (41), corresponding to the molecular formula $C_{32}H_{52}O_2$. The ^{1}H- and ^{13}C-NMR spectral data were similar to those of **1**, except the presence of an acetyl group (δ_H 2.04 and δ_C 170.28, 21.36), therefore **2** was identified as taraxeryl acetate. Its ^{1}H- and ^{13}C-NMR spectral data were identical to reported data [4].

Compound **3** was obtained as white needles. The molecular formula of **3** was established as

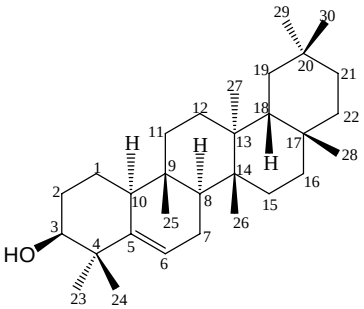
$C_{30}H_{50}O$ by combination of ^{13}C-NMR and mol peak at *m/z* 426 $[M]^+$(87). The ^{13}C-NMR spectrum showed the presence of a keton group (δ_C 212.96) and an absence of olephinic signals, suggesting that **3** is taraxeranone. In combination of its MS and NMR spectra, the structure of **3** was determined as 14α-taraxeran-3-one (3-taraxeranone) [5].



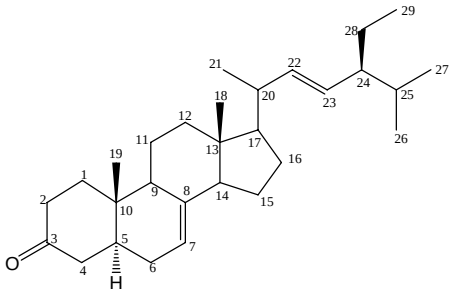
1: R = H Taraxerol
2: R = Ac Taraxeryl acetate



3:14-α-Taraxeran-3-one



4: D:B-fiedoolean-5-ene-3-β-ol



5: α-Spinasterone

The EI-MS spectrum of **4** gave a mol peak at *m/z* 426 $[M]^+$(18), indicating a same molecular formula ($C_{30}H_{50}O$) as **1**. The APT and ^{13}C-NMR spectra showed the presence of 30 carbons (CH_3x8, CH_2x10, CHx5, Cqx7). In comparison of the ^{1}H- and ^{13}C-NMR spectral data (table 2) with reported data [6], the structure of **4** was identified as D: B-friedoolean-5-ene-3-β-ol, which was isolated for the first time from *Securinega tinctorena* and its isomer (D:B-friedoolean-5-ene-3-α-ol) was found in *Euphorbia royleana* (Euphorbiaceae) [6, 7]. D:B-friedoolean-5-ene-3-β-ol is a relatively rare triterpene alcohol, which can be an intermediate in the biosynthesis of friedeline.

The molecular formula of **5** ($C_{29}H_{46}O$) was determined by combination of molecular ion peak at *m/z* 410 $[M]^+$ in EI-MS as well as its ^{13}C-NMR spectra. The ^{1}H-NMR spectrum displayed two methyl doublets at δ_H 0.82, 0.84 (each 3H, *d*, *J* = 6.4 Hz), corresponding to one isopropyl group. The mass spectrum showed a fragment at *m/z* 269 (loss of the side chain) and fragments were common to related steroids, suggested that **5** is a stigmasta-diene skeleton. This was further confirmed by the presence of three olephinic methine carbons at δ_C 116.88 (C-7), 137.92 (C-22), 129.37 (C-23) and quaternary carbon at δ_C 139.33 (C-8) in the ^{13}C-NMR spectrum. The ^{1}H-NMR spectrum showed the presence of two olephinic protons at δ_H 5.13, 5.02 with *trans* configuration (*J* = 15.0 Hz). Combination of the MS, ^{1}H- and ^{13}C-NMR spectra (tables 1&2), the structure of **5** was identified as (22*E*)-5α-stigmasta-7,22-diene-3-one (α-spinasterone), which was isolated from the heartwood of *Albizzia julibrissin* and the bark of *Acacia concinna* [8].

The presence of taraxerol as main component and its derivatives is a chemical support for the taxonomy of *Codonopsis* species used in Vietnamese traditional medicine.

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