Two New Triterpenes from the Surface Layer of *Poria cocos*

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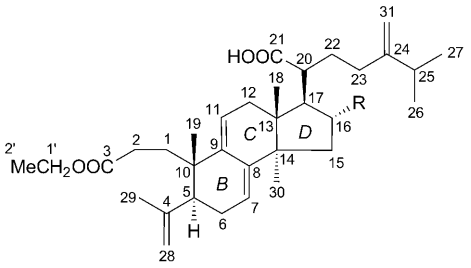
Two new triterpenes, poricoic acid AE (**1**) and poricoic acid CE (**2**), were isolated from the surface layer of the mushroom *Poria cocos* (SCHW.) WOLF, together with four known triterpenes, 3-*O*-acetyldehydroeburicoic acid, 3-oxolanosta-7,9(11),24(31)-trien-21-oic acid, dehydrotrametenolic acid, and poricoic acid A. The structures of the two new triterpenes were elucidated as 16α-hydroxy-3,4-secolanosta-4(28),7,9(11),24(31)-tetraene-3,21-dioic acid-3-ethyl ester (**1**) and 3,4-secolanosta-4(28),7,9(11),24(31)-tetraene-3,21-dioic acid-3-ethyl ester (**2**) mainly on the basis of HR-ESI-MS, EI-MS, and 2D-NMR analyses.

Introduction. – The surface layer of *Poria cocos* (SCHW.) WOLF, known as 'Fu-Ling-Pi' in Chinese folklore, was recorded in the Pharmacopoeia of P. R. China and used in many famous traditional Chinese prescriptions [1]. Modern phytochemical and pharmacological investigations showed that major active components such as triterpenoids [2] and polysaccharides [3] separated from this fungi had anti-inflammatory [4], antineoplasmic [5], and antiproliferative activities [6].

In this study, we report the isolation and structure determination of six triterpenes from the surface layer of *P. cocos*. Among them, **1** and **2** were new compounds. Their structures were elucidated on the basis of spectroscopic methods, especially EI-MS and



Poricoic acid AE (**1**) R = OH
Poricoic acid CE (**2**) R = H

2D-NMR. In addition, four known triterpenes, including 3-*O*-acetyldehydroeburicoic acid, 3-oxolanosta-7,9(11),24(31)-trien-21-oic acid, dehydrotrametenolic acid, and poricoic acid A, were also isolated from this mold and identified by comparison of their spectroscopic data with those reported in the literature, 3-*O*-acetyldehydroeburicoic acid was isolated from *P. cocos* for the first time.

Results and Discussion. – The surface layer of *P. cocos*, collected in the Anhui Province, was extracted with 95% EtOH, and then the concentrated extract suspended in water was partitioned successively with petroleum ether (PE; b.p. 60–90°), AcOEt, and BuOH. The PE and AcOEt extract was subjected to repeated column chromatography on silica gel and *Sephadex LH-20* to yield compounds **1**, **2**, and four known triterpenes.

Compound **1** was obtained as a white powder. The IR spectrum of **1** revealed the presence of an OH group (3393 cm^{-1}), an ester group (1736 cm^{-1}), a carboxyl group (1703 cm^{-1}) and a conjugated diene (1660, 1641 cm^{-1}). The HR-ESI-MS indicated the molecular formula as $C_{33}H_{50}O_5$ (*m*/*z* 525.3563 ($[M-H]^-$)), which was confirmed by ^{13}C-NMR and DEPT: seven Me, ten CH_2, and seven CH groups, and nine quaternary C-atoms. From the EI-MS, ^{1}H- and ^{13}C-NMR (*Table 1*), HMQC, HMBC (*Fig. 1* and *Table 1*) data, and NOESY (*Fig. 2* and *Table 1*) data, the structure of **1** was established as 16α-hydroxy-3,4-secolanosta-4(28),7,9(11),24(31)-tetraene-3,21-dioic acid-3-ethyl ester.

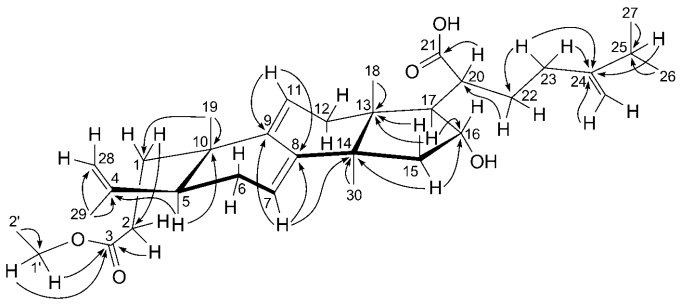


Fig. 1. *Key HMBC of* **1**

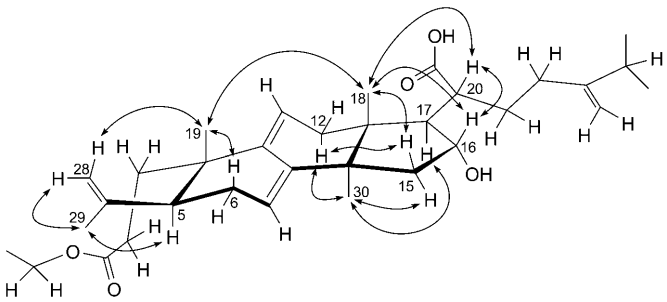


Fig. 2. *Key NOESY correlations of* **1**

Table 1. *^{1}H- and ^{13}C-NMR* ((D_5)Pyridine) *Data of* **1**. δ in ppm, J in Hz.

	δ(C)	δ(H)	HMBC (^{1}H → ^{13}C)	NOESY
H_a–C(1)	35.9	1.70–1.75 (*m*)	C(3), C(5), C(19)	–
H_b–C(1)		1.96–2.01 (*m*)	C(2), C(3), C(10)	–
H–C(2)	29.8	2.27–2.36 (*m*)	C(1), C(3), C(10), C(19)	–
C(3)	174.1	–	–	–
C(4)	149.1	–	–	–
H–C(5)	50.7	2.25–2.29 (*m*)	C(1), C(4), C(6), C(7), C(9), C(10), C(19), C(28)	Me(29)
H_α–C(6)	28.5	2.02–2.08 (*m*)	C(7), C(8)	–
H_β–C(6)		2.47–2.53 (*m*)	C(4), C(5), C(7), C(8), (9)	Me(19)
H–C(7)	118.0	5.28 (br. *s*)	C(5), C(8), C(9), C(10), C(13), C(14)	–
C(8)	141.8	–	–	–
C(9)	137.3	–	–	–
C(10)	38.8	–	–	–
H–C(11)	120.5	5.30–5.32 (*m*)	C(8), C(9), C(10), C(13)	–
H_α–C(12)	37.0	2.63–2.69 (*m*)	C(8), C(9), C(11), C(13), C(17), C(18)	H–C(17), Me(30)
H_β–C(12)		2.44–2.50 (*m*)	C(8), C(9), C(11), C(13), C(14), C(18)	–
C(13)	45.6	–	–	–
C(14)	49.3	–	–	–
H_α–C(15)	43.8	1.77–1.82 (*m*)	C(13), C(14), C(16), C(30)	Me(30)
H_β–C(15)		2.37–2.42 (*m*)	C(14), C(30)	Me(18)
H–C(16)	76.4	4.49–4.53 (*m*)	C(14), C(20)	Me(18), H–C(20)
H–C(17)	57.6	2.82–2.86 (*m*)	C(13), C(16), C(18), C(20)	H_α–C(12), Me(30)
Me(18)	18.3	1.08 (*s*)	C(12), C(13), C(14), C(17)	H_β–C(15), H–C(16), Me(19), H–C(20)
Me(19)	22.2	0.97 (*s*)	C(1), C(5), C(9), C(10)	H_β–C(6), Me(18), H_b–C(28)
H–C(20)	48.4	2.91–2.96 (*m*)	C(17), C(21), C(22)	H–C(16), Me(18)
C(21)	178.5	–	–	–
H_a–C(22)	31.4	2.42–2.46 (*m*)	C(20), C(23)	–
H_b–C(22)		2.63–2.67 (*m*)	C(23)	–
H_a–C(23)	33.2	2.36–2.40 (*m*)	C(22), C(24), C(31)	–
H_b–C(23)		2.50–2.54 (*m*)	C(22), C(24), C(31)	–
C(24)	156.1	–	–	–
H–C(25)	34.1	2.25–2.30 (*m*)	C(23), C(24), C(26), C(27), C(31)	–
Me(26)	22.0	1.00 (*d*, J = 6.8)	C(24), C(25), C(27)	–
Me(27)	21.9	0.98 (*d*, J = 6.8)	C(24), C(25), C(26)	–
H_a–C(28)	112.2	4.76 (br. *s*)	C(5), C(29)	–
H_b–C(28)		4.81 (br. *s*)	C(5), C(29)	Me(19), Me(29)
Me(29)	22.1	1.72 (*s*)	C(4), C(5), C(28)	H–C(5), H_b–C(28)
Me(30)	24.8	1.43 (*s*)	C(8), C(13), C(14), C(15)	H_α–C(12), H_α–C(15), H–C(17)
H_a–C(31)	107.1	4.85 (*s*)	C(23), C(24), C(25)	–
H_b–C(31)		4.98 (*s*)	C(23), C(24), C(25)	–
CH_2(1′)	60.2	4.12 (*q*, J = 7.1)	C(3), C(2′)	–
Me(2′)	14.3	1.14 (*t*, J = 7.1)	C(1′)	–

The EI-MS of **1** showed a molecular-ion peak at 526 (M^+), and gave a base peak at m/z 425 ($[M - CH_2CH_2COOC_2H_5]^+$) derived from the cleavage at the C(1)–C(10) bond from M^+. A significant peak at m/z 407 ($[M - CH_2CH_2COOC_2H_5 - H_2O]^+$), derived from the cleavage at the C(16)–OH bond from $[M - CH_2CH_2COOC_2H_5]^+$, indicated that **1** was a 3,4-secolanostane triterpene with a OH substituent at C(16) [7]. The IR spectrum (KBr) displayed absorption bands for a OH group (3393 cm^{-1}), which confirmed this assumption.

The ^{13}C-NMR spectrum of **1** showed signals of two olefinic CH groups at δ(C) 118.0 and 120.5 (C(7) and C(11)) and two olefinic quaternary C-atom resonances at δ(C) 141.8 and 137.3 (C(8) and C(9)). The long-range correlation of H–C(7)/C(8), H–C(7)/C(9), and H–C(11)/C(9), and H–C(11)/C(8) observed in the HMBC experiment revealed the presence of the C(7)=C(8) and C(9)=C(11) system, which was confirmed by the UV absorption at 243 nm [7]. The correlation of H–C(5)/H–C(6), H–C(6)/H–C(7) in the ^{1}H,^{1}H-COSY spectrum and H–C(5)/C(10), H–C(5)/C(9), H–C(7)/C(8), and H–C(7)/C(9) in the HMBC spectrum indicated the existence of the six-membered ring *B:* C(5)–C(6)–C(7)–C(8)–C(9)–C(10). Similarly, the five-membered ring *D* C(13)–C(14)–C(15)–C(16)–C(17) was confirmed by the correlation of H–C(15)/H–C(16) and H–C(16)/H–C(17) in the ^{1}H,^{1}H-COSY spectrum, as well as H–C(15)/C(14), H–C(15)/C(13), and H–C(17)/C(13) in the HMBC experiment. The connection of C(12)–C(13) and C(8)–C(14) was deduced from the HMBC cross-peaks of H–C(12)/C(11), H–C(12)/C(13), H–C(17)/C(13), and H–C(7)/C(14), H–C(15)/C(14), thus, C(8)–C(9)–C(11)–C(12)–C(13)–C(14) was determined as the six-membered *C* ring of the lanostane skeleton.

The ^{1}H-NMR spectrum of **1** revealed four *singlet*s at δ(H) 0.97, 1.08, 1.43, 1.72 due to the four Me groups of the 3,4-secolanostane skeleton; assigned to Me(19), Me(18), Me(30), and Me(29) by the HMBC experiment, respectively. In the ^{1}H,^{1}H-COSY spectrum, the correlations of H–C(15)/H–C(16), H–C(16)/H–C(17), H–C(17)/H–C(20), H–C(20)/H–C(22), and H–C(22)/H–C(23) indicated the connectivity of C(15)–C(16)–C(17)–C(20)–C(22)–C(23); in the ^{1}H-NMR, two *doublet*s at δ(H) 1.00, 0.98 and a *mutiplet* at δ(H) 2.25–2.30, which were coupled to each other, were attributed to ($(Me)_2$CH–C(24)) by the long-range correlation of H–C(25)/C(24) in the HMBC spectrum; with the HMBC cross peaks of H–C(23)/C(24), H–C(31)/C(24), and H–C(20)/C(21), the existence of the C(20, 21)–C(22)–C(23)–C(24,31)–C(25)–C(26,27) side chain of the 3,4-secolanostane structure was confirmed. A *triplet* at δ(H) 1.14 (J = 7.1) and a *quadruplet* at δ(H) 4.12 (J = 7.1), which were coupled to each other, were attributed to the $MeCH_2O$–C(3)=O group as confirmed by the HMBC of H–C(1′)/C(3). The presence of an isopropenyl group was suggested by a Me group (Me(29)–C(4)) at δ(H) 1.72 and an exomethylene group at δ(H) 4.76 and 4.81, and the C(4)–C(5) connection was confirmed by the HMBC cross-peak of H–C(5)/C(4). The above data strongly suggested that **1** was a 16-hydroxy-3,4-secolanostane. Comparison of the ^{13}C-NMR data with those of the known compound poricoic acid A [8] revealed that the signals were very similar except for the additional signal of the EtO group. Accordingly, the structure of compound **1** was established as 16-hydroxy-3,4-secolanosta-4(28),7,9(11),24(31)-tetraene-3,21-dioic acid-3-ethyl ester.

The relative configuration of **1** was determined mainly by NOESY (*Fig. 2*) analyses, with major NOE signals between Me(18)/Me(19), Me(18)/H_β–C(16), and

Me(30) /H_α–C(17); the OH group connected with C(16) was in α-orientation, thus **1** was confirmed as 16α-hydroxy-3,4-secolanosta-4(28),7,9(11),24(31)-tetraene-3,21-dioic acid-3-ethyl ester, named poricoic acid AE.

Compound **2** was obtained as a white powder. The IR spectrum of **2** revealed the presence of a OH group (3425 cm^{-1}), an ester group (1738 cm^{-1}), a carboxyl group (1715 cm^{-1}), and a conjugated diene (1659, 1641 cm^{-1}). By HR-ESI-MS, the molecular formula was determined to be $C_{33}H_{50}O_4$ (*m/z* 509.3615 ($[M-H]^-$)). From the EI-MS, ^{1}H- and ^{13}C-NMR (*Table 2*), HMQC, HMBC (*Fig. 3* and *Table 2*), and NOESY (*Fig. 4* and *Table 2*) data, the structure of **2** was established as 3,4-secolanosta-4(28),7,9(11),24(31)-tetraene-3,21-dioic acid-3-ethyl ester.

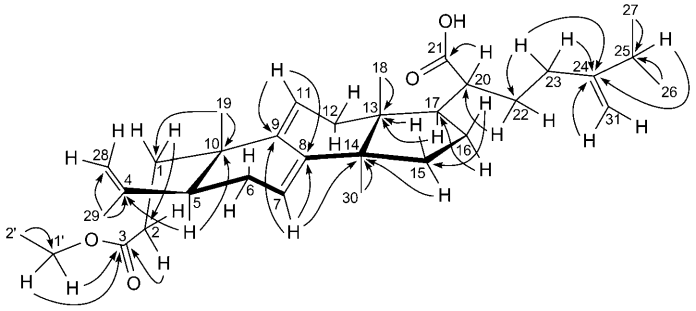


Fig. 3. *Key HMBC of* **2**

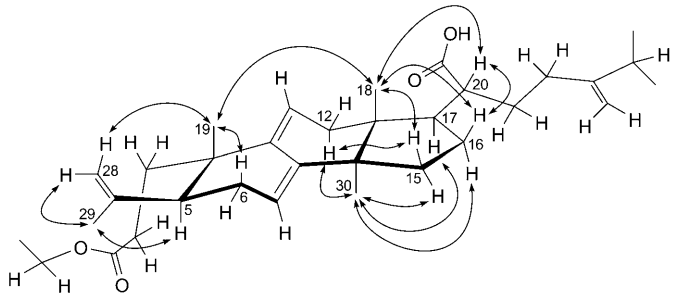


Fig. 4. *Key NOESY correlations of* **2**

The EI-MS of **2** showed a molecular-ion peak at 510 (M^+), and gave a base peak at *m/z* 409 ($[M-CH_2CH_2COOC_2H_5]^+$) derived from the cleavage at the C(1)–C(10) bond from M^+; no peak was observed at *m/z* 390 ($[M-CH_2CH_2COOC_2H_5-H_2O]^+$), which confirmed that **2** was a 3,4-secolanostane triterpene without C(16)–OH substitution [7]. The UV absorption of **2** at 243 nm suggested the presence of conjugated C=C bonds, which indicated the C(7)=C(8) and C(9)=C(11) moiety [7]. The IR spectrum (KBr) also displayed absorption bands for a conjugated diene moiety (1659, 1641 cm^{-1}). The ^{1}H-NMR and ^{13}C-NMR (DEPT) spectra of **2** revealed signals due to seven Me, eleven CH_2, and six CH groups, as well as nine quaternary C-atoms.

Table 2. *^{1}H- and ^{13}C-NMR* ((D_5)Pyridine) *Data of* **2**. δ in ppm, J in Hz.

	δ(C)	δ(H)	HMBC (^{1}H → ^{13}C)	NOESY
H_a–C(1)	35.9	1.68–1.74 (*m*)	C(2), C(3), C(5), C(9), C(10)	–
H_b–C(1)		1.96–2.02 (*m*)	C(2), C(3), C(10)	–
H_a–C(2)	29.9	2.25–2.32 (*m*)	C(1), C(3), C(10), C(19)	–
H_b–C(2)		2.33–2.37 (*m*)	C(1), C(3)	–
C(3)	174.1	–	–	–
C(4)	149.1	–	–	–
H–C(5)	50.8	2.25–2.29 (*m*)	C(1), C(4), C(6), C(7), C(9), C(10), C(19), C(28), C(29)	Me(29)
H_α–C(6)	28.6	2.03–2.08 (*m*)	C(4), C(7), C(8), C(10)	–
H_β–C(6)		2.48–2.55 (*m*)	C(5), C(9)	Me(19)
H–C(7)	118.0	5.27 (br. *s*)	C(8), C(9)	–
C(8)	141.8	–	–	–
C(9)	137.3	–	–	–
C(10)	38.8	–	–	–
H–C(11)	120.6	5.28 (br. *s*)	C(8), C(9)	–
H_α–C(12)	36.8	2.46–2.49 (*m*)	C(9), C(11), C(13), C(14), C(18)	H–C(17), Me(30)
H_β–C(12)		2.44–2.46 (*m*)	C(9), C(11), C(13), C(14), C(18)	–
C(13)	44.7	–	–	–
C(14)	50.3	–	–	–
H_α–C(15)	31.0	1.37–1.42 (*m*)	C(13), C(14), C(30)	Me(30)
H_β–C(15)		1.72–1.77 (*m*)	C(8), C(14), C(16), C(30)	Me(18)
H_α–C(16)	27.2	1.42–1.47 (*m*)	C(13), C(14), C(17)	Me(30)
H_β–C(16)		2.06–2.11 (*m*)	C(15)	Me(18), H–C(20)
H–C(17)	48.3	2.46–2.52 (*m*)	C(13), C(16), C(18), C(20)	H_α–C(12), Me(30)
Me(18)	17.0	1.01 (*s*)	C(12), C(13), C(14), C(17)	H_β–C(15), H_β–C(16), Me(19), H–C(20)
Me(19)	22.1	0.96 (*s*)	C(1), C(5), C(9), C(10)	H_β–C(6), Me(18), H_b–C(28)
H–C(20)	48.9	2.64 (*td*, J = 11.0, 3.2)	C(21)	H_β–C(16), Me(18)
C(21)	178.3	–	–	
H_a–C(22)	31.7	2.25–2.30 (*m*)	C(23), C(24), C(31)	–
H_b–C(22)		2.38–2.43 (*m*)	C(20), C(23), C(24),	–
H_a–C(23)	32.7	2.25–2.30 (*m*)	C(22), C(24), C(31)	–
H_b–C(23)		2.38–2.43 (*m*)	C(20), C(22), C(24), C(31)	–
C(24)	155.9	–	–	–
H–C(25)	34.2	2.25–2.30 (*m*)	C(24), C(26), C(27), C(31),	–
Me(26)	21.9	1.02 (*d*, J = 6.8)	C(24), C(25), C(27)	–
Me(27)	22.0	1.03 (*d*, J = 6.8)	C(24), C(25), C(26)	–
H_a–C(28)	112.2	4.75 (br. *s*)	C(5), C(29)	–
H_b–C(28)		4.80–4.81 (*m*)	C(5), C(29)	Me(19), Me(29)
Me(29)	22.1	1.71 (*s*)	C(4), C(5), C(10), C(28)	H–C(5), H_b–C(28)
Me(30)	24.3	0.99 (*s*)	C(8), C(13), C(14), C(15)	H_α–C(12), H_α–C(15), H_α–C(16), H–C(17)
H_a–C(31)	107.0	4.89 (br. *s*)	C(23), C(24), C(25)	–
H_b–C(31)		4.93 (br. *s*)	C(23), C(24), C(25)	–
CH_2(1′)	60.2	4.15 (*q*, J = 7.1)	C(3), C(2′)	–
Me(2′)	14.3	1.17 (*t*, J = 7.1)	C(1′)	–

Comparison of the ^{13}C-NMR data with those of the known compound poricoic acid C [9] revealed that the signals were very similar, except for the additional signals of an existing EtO group. Furthermore, the ^{13}C-NMR data of **2** were also very similar to **1**, except for the signal of C(16) (CH group replaced by a CH_2 group, as observed from the DEPT experiment) which was shifted from 76.4 ppm to relatively high field (27.2 ppm).

Thus, **2** was deduced as 3,4-secolanosta-4(28),7,9(11),24(31)-tetraene-3,21-dioic acid-3-ethyl ester, named poricoic acid CE.

The four known triterpenoids 3-*O*-acetyldehydroeburicoic acid [10], 3-oxolanosta-7,9(11),24-trien-21-oic acid [11], dehydrotrametenolic acid [9], and poricoic acid A [8], were also isolated from *Poria cocos*. The structures were identified by comparison of their spectroscopic data (ESI-MS, ^{1}H- and ^{13}C-NMR) with those reported in the literature. 3-*O*-Acetyldehydroeburicoic acid was isolated from *P. cocos* for the first time.

The authors are grateful to Prof. *Wei-Chun Wu* (Shenyang Pharmaceutical University) for collecting and identification of the materials.

Experimental Part

General. Column chromatography (CC): Silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Plant*, Qingdao, P. R. China); *Sephadex LH-20* (*Pharmacia*). TLC: silica gel plates (*Qingdao Marine Chemical Plant*, Qingdao, P. R. China). M.p.: *XT-4* micro-melting-point apparatus; uncorrected. Optical rotations: *Perkin-Elmer MC-241* polarimeter. UV Spectra: *Shimadzu UV-2401* PC spectrophotometer; λ_{max} in nm. IR Spectra: *Nicolet Impact 410* FT-IR spectrometer; KBr pellets; in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker AV-500* spectrometer; δ in ppm, J in Hz. MS: *Agilent 1100-LC/TOF MSD* (HR-ESI/MS), and *Shimadzu GC-T2010* (EI-MS); in m/z.

Plant Material. The outer layers of *Poria cocos* were collected in May 2006 from Bozhou County of Anhui Province, P. R. China and identified by Prof. *Wei-Chun Wu* (Shenyang Pharmaceutical University). A voucher specimen (No. 20060910) was deposited with the Key Laboratory of Drug Quality Control and Pharmacovigilance, Ministry of Education, China Pharmaceutical University, Nanjing, P. R. China.

Extraction and Isolation. The powdered surface layer of *Poria cocos* (5.0 kg) was repeatedly extracted with 95% EtOH at r.t. for three times. The extract was then concentrated under reduced pressure to give a brown syrup, which was partitioned into petroleum ether (PE)-soluble (30.5 g), AcOEt soluble (58.4 g), and BuOH-soluble (18.6 g) fractions. The PE-soluble fraction was subjected to CC (SiO_2, $CHCl_3 \rightarrow CHCl_3$/MeOH 2:1): *Fractions A–K. Fr. D* (4.7 g) was resubmitted to CC (SiO_2, $CHCl_3$/MeOH 20:1 → 2:1): *Fr. D.1–D.5*. *Fr. D.2* (257 mg) was further purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1): **3** (14 mg), *Fr. D.4* (2.2 g) was separated by CC (SiO_2, $CHCl_3$/MeOH 5:1): *Frs. D.4.1* and *D.4.2. Fr. D.4.1* was further purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1): **2** (25 mg), *Fr. D.4.2* was also purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1): **4** (30 mg). The AcOEt soluble fraction was subjected to CC (SiO_2, $CHCl_3$/MeOH 10:1 → 1:2): *Frs. L–T. Fr. N* (3.9 g) was resubmitted to CC (SiO_2, $CHCl_3$/MeOH 5:1 → 2:1): *Frs. N.1–N.6. Fr. N.5* (130 mg) was further purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1): **5** (15 mg), *Fr. N.6* (110 mg) was further purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1): **1** (12 mg), *Fr. P* (1.1 g) was separated by CC (SiO_2, $CHCl_3$/MeOH 1:1): *Frs. P.1* and *P.2. Fr. P.1* (125 mg) was purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1): **6** (11 mg).

16α-Hydroxy-3,4-secolanosta-4(28),7,9(11),24(31)-tetraene-3,21-dioic Acid-3-ethyl Ester (=*2-[*rel-*(2*R*,3*R*,3a*R*,6*S*,7*S*,9b*R*)-6-(3-Ethoxy-3-oxopropyl)-2,3,3a,4,6,7,8,9b-octahydro-2-hydroxy-3a,6,9b-trimethyl-7-(prop-1-en-2-yl)-1*H*-cyclopenta[a]naphthalen-3-yl]-6-methyl-5-methylideneheptanoic Acid*; **1**). White powder. M.p. 215–216° ($CHCl_3$). $[\alpha]_D^{25} = +20.5$ ($c = 0.6$, $CHCl_3$). IR (KBr): 3393, 3075, 2964, 2924, 2893, 2853, 1767, 1736, 1703, 1660, 1641, 1452, 1375, 1288, 1250, 1196, 1180, 1026, 893. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 526 (7, M^+), 508 (4), 481 (5), 441 (9), 425 (100, $[M - CH_2CH_2COOC_2H_5]^+$),

407 (18, $[M - CH_2CH_2COOC_2H_5 - H_2O]^+$), 377 (4), 353 (3), 329 (3), 251 (12), 211 (15), 197 (17), 183 (16), 171 (18), 159 (30), 145 (55), 131 (20), 119 (21), 105 (21). HR-ESI-MS: 525.3563 ($[M - H]^-$, $C_{33}H_{49}O_5^-$; calc. 525.3580).

3,4-Secolanosta-4(28),7,9(11),24(31)-tetraene-3,21-dioic Acid-3-ethyl Ester (=*2-[*rel-*(3*R*,3a*R*,6*S*,7*S*,9b*R*)-6-(3-Ethoxy-3-oxopropyl)-2,3,3a,4,6,7,8,9b-octahydro-3a,6,9b-trimethyl-7-(prop-1-en-2-yl)-1*H*-cyclopenta[*a*]naphthalen-3-yl]-6-methyl-5-methylideneheptanoic Acid*; **2**). White powder. M.p. 201–203° ($CHCl_3$). $[\alpha]_D^{25} = +27.5$ ($c = 0.5$, $CHCl_3$). IR (KBr): 3425, 3072, 2962, 2926, 1738, 1715, 1659, 1641, 1452, 1375, 1300, 1221, 1184, 1032, 891. ^{1}H- and ^{13}C-NMR: *Table 2*. EI-MS: 510 (3, M^+), 437 (10), 409 (100, $[M - CH_2CH_2COOC_2H_5]^+$), 309 (10), 295 (8), 253 (10), 225 (8), 211 (9), 197 (10), 183 (12), 169 (14), 157 (28), 145 (60), 129 (35), 119 (36), 105 (45), 96 (70), 81 (75), 69 (80), 55 (85). HR-ESI-MS: 509.3615 ($[M - H]^-$, $C_{33}H_{49}O_4^-$; calc. 509.3631).

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