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New hydroquinone diglycoside acyl esters and sesquiterpene and apocarotenoid from *Ecdysanthera rosea*

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ABSTRACT

Phytochemical studies on the ethanol extract of the aerial parts of *Ecdysanthera rosea* led to the isolation of three new compounds, hydroquinone diglycoside acyl esters, ecdysanrosin A (1) and sesquiterpene, 5β-hydroperoxycostic acid (2) and apocarotenoid, 2, 4, 7-trimethyl-2, 4, 6, 8-tetraene-dialdehyde (3). Their structures were elucidated on the basis of extensive spectroscopic analysis.

1. Introduction

Ecdysanthera rosea Hook. et Arn. (Apocynaceae) is a large climbing shrub scattered in tropical Asia, which has been used as anti-inflammatory, antibacterial, antipyretic, anti-hepatitis agent and had diuretic activity [1]. A literature search revealed that some compounds such as ecdysantherin, 20-Epi-Kibataline, 3β,14β-,20-trihydroxi-18oic (18–20) lactone pregnen-5 [2], 5-O-caffeoylquinic derivers, scopoletin, tartaric acid, malic acid, phytosterol, triterpenoid, and saponine [3], D-friedours-14-en-11α,12α-epoxy-3β-yl palmitate [4] have been isolated from this plant. As part of further phytochemical and pharmacological investigations into the genus *Ecdysanthera*, we collected the stems of *E. rosea* from Xishuangbanna in Yunnan province. From the EtoAc extract, a new hydroquinone diglycoside acyl esters, ecdysanrosin A (1) and sesquiterpene, 5β-hydroperoxycostic acid (2) and apocarotenoid, 2, 4, 7-trimethyl-2, 4, 6, 8-tetraene-dialdehyde (3) with several known compounds, namely, (tianshic acid) (4), ent-isolariciresinol (5), (−)-2α-O-(β-D-Glucopyranosyl) lyoniresinol(6), (+)-lyoniresinol(7), 1β, 6α-dihydroxy-4 (14) eudesmene (8), have been isolated. Herein, details of the isolation and structure elucidation of compounds 1–3 are described.

2. Experimental

2.1. General

Optical rotations were obtained on a Horiba SEPA-300 polarimeter. UV Spectra were obtained on a Shimadzu 210A double-beam spectrophotometer, λ_{max} in nm. IR spectra were taken on a Bio-Rad FTS-135 infrared spectrophotometer with KBr pellets. 1D and 2D-NMR spectra were recorded on Bruker AM-400 and DRX-500 instruments with TMS as internal standard, δ in ppm, J in Hz. EI-MS, ESI-MS and HR-ESI-MS were measured on Finnigan-MAT 90 and API QSTAR Pulsarimass spectrometers, respectively. Silica gel 200–300 mesh (Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Fractions were monitored by TLC (Qingdao Marine Chemical Inc., China) TLC spots were detected by spraying with 10% H_2SO_4 in EtOH followed by heating.

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2.2. Plant material

Aerial parts of *E. rosea* were collected at Xishuangbanna, Yunnan province, China, in May, 2004.The plant was identified by Dr. Li Rong, Kunming Institute of Botany, Chinese Academy of Sciences. A sample (Kun No. 20040501) has been deposited in Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, PR China.

2.3. Extraction and isolation

Dried stems and leaves of *E. roesa* (11 kg) were extracted three times with 90% ethanol at reflux, 2 h one time, and the extract was filtered. After evaporation of ethanol in vacuo, the concentrated extract was suspended in H_2O and extracted with petroleum ether, EtOAc and n-BuOH. The EtOAc extract (79 g) was subjected to CC (silica gel (200–300 mesh), $CHCl_3$/MeOH (95:5–7:3): Seven fractions were obtained by monitoring with TLC (F1–7). F4 (8 g) was repeatedly chromatographed over silica gel with $CHCl_3$-MeOH from 95:5 to 90:10 and purified on sephadex-LH-20, eluted with $CHCl_3$-MeOH 1:1, and then on reverse phase chromatography (RP-18) eluted with CH_3OH-H_2O (from 4: 6 to 8:2) to afford **4**, **5**,**7**, **8**. F5 (22 g) was repeatedly chromatographed over silica gel, eluted with $CHCl_3$-MeOH 95:5–85:15, and purified on sephadex-LH-20, eluted with $CHCl_3$-MeOH 1:1 and then on reverse phase chromatography (RP-18) eluted with CH_3OH: H_2O 5: 5 affording **1**,**2**, **3**, **7**.

Ecdysanrosin A (1), amorphous powder, $[\alpha]_D^{24.8} = -49.7°$(*c* 0.99, MeOH), UV: λ_{max} (nm) (MeOH): 206, 280; IR(KBr) cm^{-1} : 3425, 2938, 1703, 1612, 1514, 1284, 1244, 1111, 1070, 1029, 763; ^1H and ^{13}C NMR data see Table 1. Negative FAB-MS *m/z* 583[M–H]$^-$, HREI-MS *m/z*: 583.1652 ($C_{26}H_{31}O_{15}$, calc 583.1662) (Table 2).

Table 1
NMR spectral data for compound 1(CD_3OD) and Seguinoside K(CD_3OD).

C	Seguinoside	1	H	Seguinoside K	1
1	152.5	152.6			
2	103.1	103.8	2	6.66(d, 2)	6.68(d, 2)
3	149.2	149.1			
4	142.7	142.9			
5	116.0	116.0	5	6.54(d, 8)	6.64(d, 8)
6	109.4	109.8	6	6.44(dd, 8, 2)	6.56(dd, 8, 2)
1	101.9	103.6	1`	4.79(d, 8)	4.69(d, 8)
2	78.9	78.6	2`	4.05(d, 1)	
3	78.5	74.8			
4	71.7	71.5			
5	78.8	76.7			
6	62.7	68.4	6 a	3.66(dd, 12, 6)	3.60(m)
			6 b	3.88(dd, 12 , 2)	4.06(dd, 12, 2)
1″	110.6	110.5	1″	5.50(d, 1)	5.01(d, 2.0)
2″	78.1	77.8			
3″	79.3	79.0	4″a	3.90(d, 10)	4.08(d, 10)
4″	75.7	74.9	4″b	4.30(d, 10)	3.86(d, 10)
5″	68.0	67.6	5″a	4.29(d, 11)	4.35(d, 11)
COO	167.8	167.8	5″b	4.39(d, 11)	4.33(d, 11)
1″	122.3	122.0			
2″	113.8	113.7	2″	7.47(d, 2)	7.54(d, 2)
3″	153.0	152.6			
4″	148.7	148.9			
5″	125.3	125.3	5″	6.78(d,8)	6.85(d, 8)
6″	115.9	116.0	6″	7.50(dd, 8, 2)	7.56(dd, 8, 2)
OMe	56.3	56.4	3-OMe	3.73(s)	3.76
OMe	56.3	56.8	3″ OMe	3.83(s)	3.84

Table 2
NMR spectral data for compound 2(CD_3OD) and 9($CDC1_3$).

2					9
No	δ_c	No	δ_H	HMB C(H ~ C)	δ_H
1	38. 1t	1α	1.16 m	C-2, 3, 10	
		1β	1.82 dt	C-2, 10	
2	23. 3t	2	1.68–1.60 m	C-1,3	
3	34. 1t	3α	2.51 m		2.49dtt
		3β	2.48 m	C-4, 5	2.18ddd
4	146.8s				
5	87. 3s				
6	34. 2t	6α	2.13 dd	C-5, 7	2.08brdd
		6β	1.57 m	C-4, 5, 15	1.63t
7	37. 6d	7	2.83 m	C-6, 11, 13	2.88brtt
8	27. 5t	8	1.60 m	C-7	1.5–1.7 m
9	35. 6t	9α	1.91 m	C-8, 10	1.5–1.7 m
		9β	1.03 m	C-8, 10	1.14 m
10	39.9 s				
11	147.9 s				
12	171.2s				
13	122. 1t	13α	6.11 brs	C-7, 11, 12	6.18brs
		13β	5.56brs	C-7, 11, 12	5.60brs
14	23.0q	14	1.05s	C-1, 2, 5, 8, 9, 10	1.07s
15	115.5t	15α	5.30brs	C-3, 4, 5	5.27brs
		15β	5.17brs	C-3, 5	5.01brs
OMe					3.77s

5β-hydroperoxycostic acid (2), colourless solid, $[\alpha]_D^{24.8} = -6.3°$(*c* 0.56, MeOH), UV λ_{max} (nm) (MeOH): 207, 313 nm, IR (KBr) cm^{-1}: 3431, 2931, 2869, 1705, 1625, 1381, 1261, 1032 cm^{-1}; ^1H and ^{13}C NMR data see Table 1. Negative FAB-MS *m/z*: 265[M–H]$^-$, HREI-MS *m/z*: 265.1441($C_{15}H_{21}O_4$, calc 265.1439).

2, 4,7 -trimethyl-2, 4, 6, 8-tetraene-dialdehyde (3), colourless oil, ^1H and ^{13}C NMR data see Table 3. Negative FAB-MS *m/z*: 251[M–H]$^-$.

3. Results and discussion

Ecdysanrosin A (1) was isolated as an amorphous powder, and its elemental composition was revealed as $C_{26}H_{31}O_{15}$ by negative ion HR-FAB mass spectrometry (found 583.1652 calcd. 583.1662). The negative FAB mass spectrum showed peak at *m/z* 583 [M–H]$^-$. Absorption maxima at 206 and 280 nm in the UV spectrum and absorption at, 1612 and 1514 cm^{-1} in the IR

Table 3
NMR spectral data for compound 3(CD_3OD).

No	δ_c	δ_H	HMB C(H–C)
1	192.8 d	9.72(s)	2, 3
2	152.3 s		
3	112.2 d	7.23(d, 1.3)	2, 4
4	149.6 s		
5	125.1 d	7.15(dd, 8.2, 1.3)	4, 6, 7
6	166.6 d	6.84(d, 8.2)	5, 7
7	149.5 s		
8	156.6 d	7.56(d, 15.7)	7, 9
9	122.6 d	6.63(dd,15.7,7.8)	8, 10
10	196.1 d	9.55(d, 7.8)	9
OMe-2	56.5 q	3.89(s)	2
OMe-4	56.5 q	3.89(s)	4
OMe-7	56.8 q	3.88(s)	7

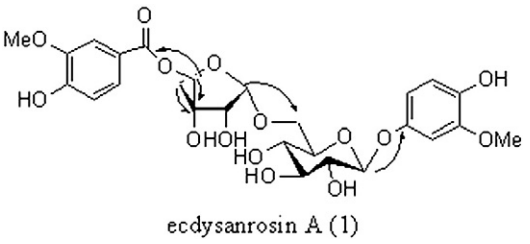

Fig. 1. Key HMBC correlations of compound **1**.

spectrum suggested the presence of an aromatic protons. The ^{1}H NMR spectrum showed the presence of two sets of three aromatic protons coupled in an ABX sytem and two methoxyl signal. The ^{1}H NMR and HSQC spectra of **1** exhibited, in the aromatic region, a set of signals at δ 6.68(1H, d, J=2 Hz, H-2), 6.64(1H, d, J=8 Hz H-5), 6.56(1H, dd, J=8, 2 Hz, H-6) and another set of signals at δ 7.54(1H, d, J=2 Hz, H-2″), 6.85(1H, d, J=8 Hz H-5″), 7.56(1H, dd, J=8, 2 Hz, H-6″), two methoxy signals at 3.84 and 3.76. The ^{13}C NMR spectrum indicated that the sugar portion was the same as that of β-apioforanose and β-glucopyranoside of seguioside K [5], and the acyl moiety is also the same as that of seguioside K. Anomeric proton signals at δ 4.69 (1H, d, 8 Hz) of glucose indicating the presence β-linkage. The signal correlated at δ 5.01 (H-1″) with δ 68.4 (C-6′) showed the β-apioforanosyl unit is affixed to C-6′ of β-glucopyranoside by the HMBC spectrum experimental (see Fig. 1). Therefore compound **1** was elucidated as shown in Fig. 2, named ecdysanrosin A.

5β-hydroperoxycostic acid (2) colourless oil, $[\alpha]_D^{24.8} = -6.3°$(*c* 0.56, MeOH), has a molecular formula of $C_{15}H_{21}O_4^-$, as established by HR-ESIMS (found 265.1441 calcd. 265.1439). The negative FAB mass spectrum showed peak at *m/z* 265 [M–H]$^-$. IR spectrum exhibited absorption maxima at 207 and 313 nm in the UV spectrum and absorption at 3431(OH), 1705 (CO), 1625(C=C) cm^{-1}. The ^{13}C NMR spectrum revealed 15 signals: these were sorted by DEPT experiments into $CH_3\times1$, $CH_2\times6$, $CH\times1$, $=CH_2\times2$ and $C\times5$ (Table 1). The ^{1}H NMR spectrum showed the chemical shift of a methyl group being δ_H1.05 (3H, s, H-14), two methylene δ_H 6.11(brs, H-13α), 5.56 (brs H-13β), 5.30(brs H-15α) 5.17(brs H-15β) and a hypo-

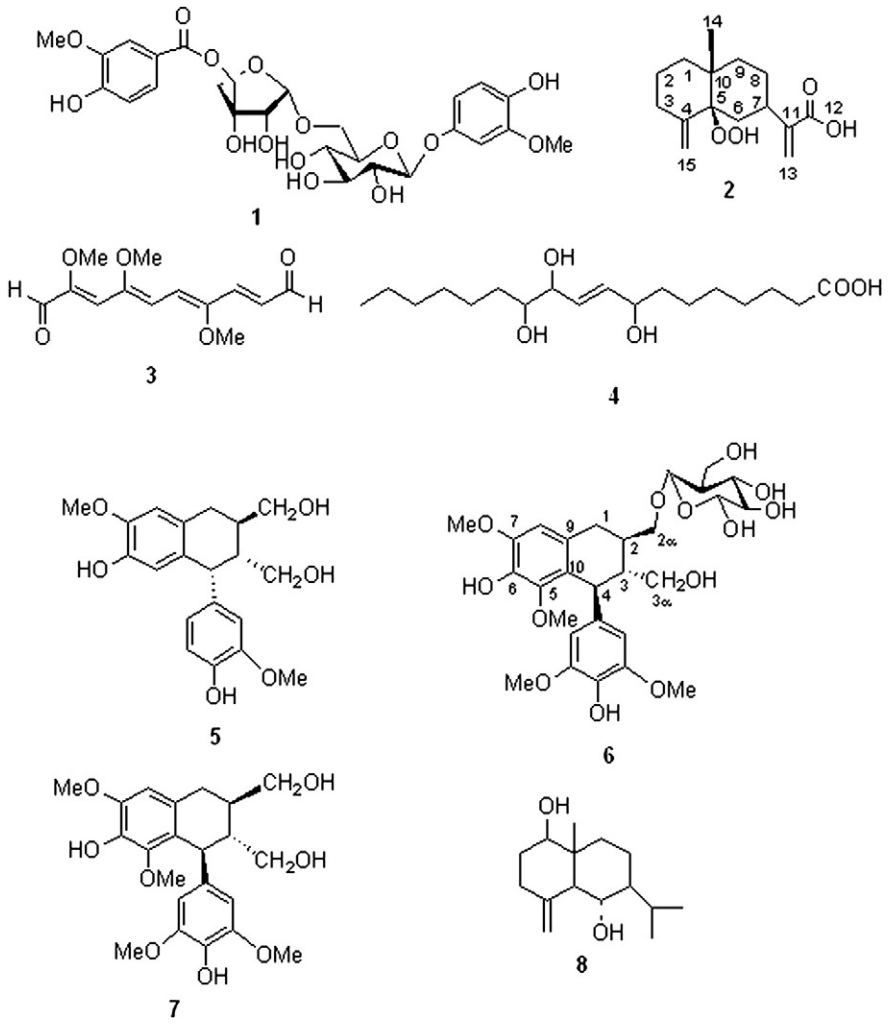

Fig. 2. Structures of compounds 1–8.

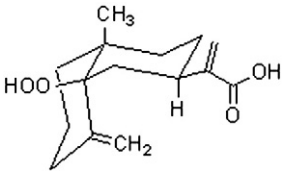

Fig. 3. The cis ring fusion hdroperoxide 2.

methyl δ_H 2.85(1H, m, H-7). Compared with the literature, the ^{1}H NMR spectrum (Table 1) is very similar to that of 5β-hydroxy costic acid methyl ester (**9**)[6]. The ^{13}C NMR spectrum exhibited the main difference being low-field shifts (+12) of the signals of C-5, compared with 5α-hydroxy costic acid [7]. The HR-ESIMS spectrum of **2** gives evidence supporting the presence of a peroxide group. The position of two double bonds was confirmed by HMQC and HMBC experimental. The beta-configuration for the hydroperoxyl group was established according to these data on hydroperoxytelekin [8] and compared with the costic acid derivative. The signals of H-14, H-7 H-15 appear at a lower field than those of 5α-hydroxy costic acid, as expected from the deshielding effect produced on these hydrogen atoms by the equatorial 5-OOH and double band in C-4,15, C-11,13 and C-12, O, respectively. This suggests that the cis form ring fusion of hydroperoxide (see Fig. 3). Therefore compound **2** was elucidated as 5β-hydroperoxy costic acid.

2,4,7-trimethyl-2,4,6,8-tetraene-dialdehyde (3), colourless oil, has a molecular formula of $C_{13}H_{16}O_5$, as established by negative FAB and ^{1}H ^{13}C NMR. The negative FAB mass spectrum showed peak at *m/z* 265 $[M–H]^-$. ^{13}C NMR spectrum revealed 13 signals: these were sorted by DEPT experiments into $CH_3O\times3$, $=CH\times7$, and $C\times3$ (Table 1). The ^{1}H NMR spectrum showed the chemical shift of two aldehyde groups being δ_H 9.72 (1H, s) and 9.55(1H, d, $J=7.8$ Hz) respectively. The signal at δ_H 9.72(1H, s) and 9.55(1H, d, $J=7.8$ Hz) correlated with δ_C 192.8 (d, C-1) and 196.1(d, C-10) in HMQC spectrum respectively. The couple constant 8.2 Hz between H-5 and H-6 explain the two hydrogen atoms locating trans-conformation. The two hydrogen atoms at H-8 and H-9 also located trans conformation based on *J* value of 15.7 Hz. It was suggested as an apocarotenoid based on the extended conjugation system formed by six double bands. Therefore, the structure of 3 was elucidated as shown in Fig. 2, named 2, 4, 7-trimethyl-2, 4, 6, 8-tetraene-dialdehyde.

Comparison of the spectroscopic and physical data with those published allowed us to establish the structures of known (tianshic acid) [9], ent-isolariciresinol [10], (−)-2α-O-(β-D-Glucopyranosyl) lyoniresinol [11], (+)-lyoniresinol [12], 1β, 6α-dihydroxy-4(14) eudesmene [13], respectively.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fitote.2010.06.001.

References

[1] Yunnanica Flora. Tomus 3. Institutum Botanicum Kunmingense Academiae Sinicae Edita. Beijing: Science Press; 1983.
[2] Luger P, Weber M. Dung NX. Cryst Res Technol 1998;33(2):325.
[3] Lin LC, Yang LL. Chou CJ. J Chin Med 2002;13:191.
[4] Huang KF, Sy ML, Lai JS. J Chin Chem Soc 1990;37:187.
[5] Zhong XN, Otsuka H, Ide H, Hirata E, Tkeda Y. Phytochemistry 1999;52:923.
[6] Zdero C, Bohlmann F. King KM. Phytochemistry 1990;29(10):3201.
[7] Juan FS, Enrique FJ, Alberto M. Joural of Natural Products 1990;53(4):940.
[8] James AM, Noal C, Alan RH. J Am Chem Soc 1966;88(14):3408.
[9] Chen XS, Chen DH, Si JY, Tu GA, Ma LB. Acta Pharm Sinica 2000;35(3):198.
[10] Urones JG, De Pascual T, Marcos IS, Martín DD. Phytochemistry 1987;26(5):1540.
[11] Hans A, Monika B. Ruben T. Phytochemistry 1997;45(32):325.
[12] Zhang ZZ, Guo DA, Li CL, Zheng J, Koike K, Jia C, Nikaido T. Phytochemistry 1999;51:469–72.
[13] Hu JF, Bai SP. Jia ZJ. Phytochemistry 1996;43(4):815.