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THREE NEW XANTHONES FROM THE ROOTS OF *SECURIDACA INAPPENDICULATA*

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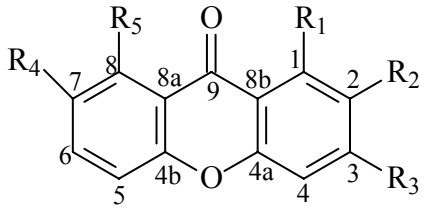
Abstract –Chemical investigation on the roots of *Securidaca inappendiculata* resulted in the isolation of three new xanthones, Securixanthones E, F and G. Their structures were characterized as 7-hydroxy-1,2-dimethoxyxanthone (**1**), 1,7-dihydroxy-2-methoxyxanthone (**2**), 3,7-dihydroxy-1,2,8-trimethoxyxanthone (**3**), respectively, on the basis of spectral evidence .

INTRODUCTION

The roots and stems of *Securidaca inappendiculata* Hassk (Polygalaceae) are used as anti-bacterial and anti-rheumatoid agents under the names “Chan yi teng”, “Wu wei teng” or “Diu le bang” in the south of the People’s Republic of China.[1] Previous phytochemical investigations have resulted in the isolation of xanthones,[2,3] xanthone glycosides,[4] a benzophenone,[5] sucrose esters[6] and organic acids[7] from the stems of this plant. In the present work, three new xanthones, Securixanthones E (**1**), F (**2**), and G (**3**) were isolated from the ethanol extract of the roots of this plant. This paper reports the isolation and structural elucidation of the compounds (**1**-**3**).



1 $R_1 = R_2 = OCH_3$, $R_3 = R_5 = H$, $R_4 = OH$
2 $R_1 = R_4 = OH$, $R_2 = OCH_3$, $R_3 = R_5 = H$
3 $R_1 = R_2 = R_5 = OCH_3$, $R_3 = R_4 = OH$

Figure1 Structures of Compounds (**1**-**3**)

RESULTS AND DISCUSSION

Compound (**1**) was isolated as yellow needles. Its molecular formula was determined as $C_{15}H_{12}O_5$ by HREIMS, showing a $[M]^+$ peak at *m/z* 272.0670 (calcd 272.0685), corresponding to ten degrees of unsaturation. The UV spectrum of **1** exhibited characteristic absorptions of a xanthone ($\lambda_{max}^{CH_3OH}$ 239, 259,

319, 384 nm). The presence of a free hydroxyl group at either C-6 or C-3 was excluded based on the lack of any change in its UV spectrum upon addition of NaOAc.[8] The ^{1}H NMR spectrum displayed resonances for two methoxyl groups (δ_H 3.85 and 3.80), a hydroxyl group (δ_H 9.86), a three-proton spin system δ_H 7.43 (1H, d, J = 9.0 Hz, H-5), δ_H 7.41 (1H, d, J = 3.0 Hz, H-8), δ_H 7.24 (1H, dd, J = 9.0, 3.0 Hz, H-6) and an AB system δ_H 7.59 (1H, d, J = 9.0 Hz, H-3) and δ_H 7.34 (1H, d, J = 9.0 Hz, H-4) of two *ortho*-protons. In the ^{13}C NMR spectrum, the signal at δ_C 60.5 was indicative of a di-*ortho*-substituted methoxyl group.[9] The positions of the substituents were confirmed through a NOESY experiment (Figure 2). As a result, it was deduced that the hydroxyl group was located at C-7 position and the methoxyl groups were at C-1 and C-2 or C-3 and C-4 positions. The presence of a 1,2-dimethoxyl moiety in **1** was supported by analysis of the ^{13}C NMR spectrum. The signals of C-8b, C-1, C-2, C-3, C-4 and C-4a of **1** appeared at essentially the same chemical shifts as those (C-8a, C-8, C-7, C-6, C-5 and C-4b) of 1-hydroxy-3,4,7,8-tetramethoxyxanthone,[10] but were very dissimilar to those (C-8b, C-1, C-2, C-3, C-4 and C-4a) of 3,4-dimethoxyxanthone.[11] Furthermore, the positions of the substituents were confirmed by the HMBC spectrum (Figure 2). Therefore, the structure of **1** was concluded to be 7-hydroxy-1,2-dimethoxyxanthone, named Securixanthone E.

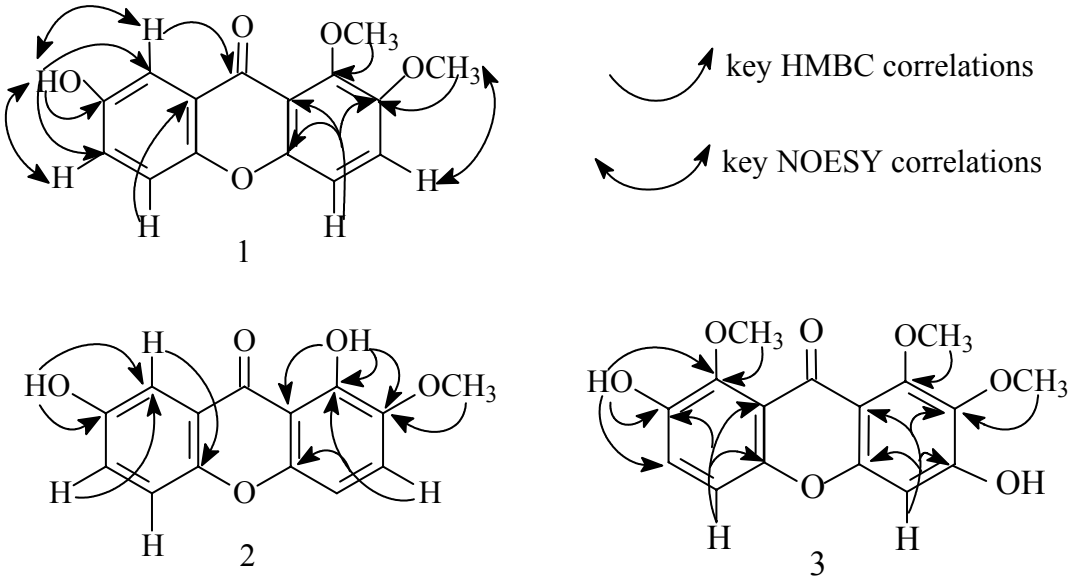


Figure 2. Key **HMBC** and **NOE** correlations of compounds (**1**-**3**)

Compound (**2**) was isolated as yellow needles. The $[M]^+$ peak at *m/z* 258.0504 (calcd 258.0528) in its HREIMS suggests a molecular formula of $C_{14}H_{10}O_5$, indicating ten degrees of unsaturation. The UV spectrum (in CH_3OH) of **2** showed absorptions at 239, 267 and 401 nm. Addition of NaOAc did not cause any change in the UV spectrum ruling out the presence of free hydroxyl groups at both C-6 and C-3. The lack of a *orhto*-dihydroxyl groups was supported by the absence of a shift after the addition of NaOAc and H_3BO_3.[8] On the basis of a comparison of the ^{1}H NMR spectral data with those of **1**,

compound (**2**) was assigned as a close derivative of **1** in which a methoxyl group is replaced by a hydroxyl group (δ_H 12.70), and the latter group appears to form intramolecular hydrogen bond to the carbonyl oxygen. In the ^{13}C NMR spectrum, the signal at δ 182.67 attributed to the carbonyl carbon was also indicative of such hydrogen bonding interactions. The hydroxyl (δ_H 10.02) was located at C-7 other than at C-6 was supported by the analysis of the ^{13}C NMR data. The C-4b, C-5, C-6, C-7, C-8 and C-8a shifts of **2** were very well agreement with those of **1**, but apparently different from those of C-4a, C-4, C-3, C-2, C-1 and C-8b of 3-hydroxy-xanthone.[12] Furthermore, the correlations in the HMBC spectrum (Figure 2) also proved compound (**2**) to be 1,7-dihydroxy-2-methoxyxanthone, which has been named Securixanthone F.

Table 1. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) Spectral Data of **1**-**3** in DMSO-d_6

	1		**2**		**3**	
Position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		147.0	12.70(s, HO)	150.1		152.9
2		148.1		142.3		138.3
3	7.59(d, 9.0)	120.2	7.54(d, 9.0)	122.5		156.8
4	7.34(d, 9.0)	112.7	7.03(d, 9.0)	106.2	6.64(s)	98.5
4a		150.0		149.9		153.0
4b		147.9		150.1		148.6
5	7.43(d, 9.0)	118.4	7.51(d, 8.5)	119.8	7.12(d, 9.0)	112.5
6	7.24(dd, 9.0, 3.0)	123.6	7.35(dd, 8.5, 3.0)	126.2	7.25(d, 9.0)	122.4
7	9.86(s, HO)	153.2	10.02(s, HO)	154.6	9.30(s, HO)	146.4
8	7.41(d, 3.0)	108.2	7.44(d, 3.0)	108.2		145.0
8a		121.6		120.1		116.8
8b		115.7		108.6		109.5
9		174.7		182.7		173.7
1-OCH_3	3.80(s, CH_3O)	60.5			3.85(s, CH_3O)	61.4
2-OCH_3	3.85(s, CH_3O)	56.2	3.79(s, CH_3O)	57.3	3.77(s, CH_3O)	60.8
8-OCH_3					3.80(s, CH_3O)	60.7

Compound (**3**) was isolated as yellow needles. Its molecular formula was revealed as $C_{16}H_{14}O_7$ by a $[M]^+$ peak at *m/z* 318.0737 (calcd 318.0739) in the HREIMS, representing ten degrees of unsaturation. The UV spectrum of **3** showed characteristic absorptions of a xanthone ($\lambda_{max}^{CH_3OH}$ 239, 286, 359 nm) and a bathochromic shift induced by the addition of NaOAc suggested the presence of a free hydroxyl group at either C-6 or C-3. The lack of *ortho*-dihydroxyl groups was substantiated by the absence of a shift after the addition of NaOAc and H_3BO_3.[8] The ^1H NMR spectrum displayed resonances for three methoxyl groups (δ_H 3.85, 3.80 and 3.77), a hydroxyl group (δ_H 9.30), an AB system (δ_H 7.12 and 7.25) of two *ortho*-coupled protons (J = 9.0 Hz) and a singlet (δ_H 6.64). Signals at δ_C 61.4, 60.8, 60.7 in the ^{13}C NMR spectrum indicated the presence of three di-*ortho*-substituted methoxyl groups.[9] The positions of the substituents were unambiguously confirmed through a HMBC experiment (Figure 2). The hydroxyl groups were located at C-3 and C-7 positions and the methoxyl groups at C-1, C-2 and C-8 positions.

Furthermore, the presence of a 3-hydroxy-1,2-dimethoxyl moiety and a 7-hydroxy-8-methoxyl moiety in **3** was supported by analysis of the ^{13}C NMR data. The chemical shifts of C-8b, C-1, C-2, C-3, C-4 and C-4a in the spectrum of **3** were comparable to those of 3-hydroxy-1,2,8-trimethoxyxanthone,[13] whilst the chemical shifts of the C-4b, C-5, C-6, C-7, C-8 and C-8a were almost in agreement with those of 1,3,7-trihydroxy-4,8-dimethoxyxanthone.[10] Therefore, the structure of **3** was confirmed as 3,7-dihydroxy-1,2,8-trimethoxyxanthone, named Securixanthone G.

EXPERIMENTAL

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and were uncorrected. UV spectra were measured on a Philips PYE Unican Pu8800 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 983G spectrometer. NMR spectra were measured in DMSO-d_6 on a Bruker AM-500 spectrometer, using TMS as internal standard. Coupling constants (*J* values) were given in Hz. A Zabspec Tofspec Platform-ESI spectrometer was used to record the EIMS and HREIMS. The TLC and HPTLC employed precoated silica gel plates (Qingdao haiyang). For the column chromatography, silica gel (Qingdao haiyang) and Sephadex LH 20 (Pharmacia) were used. MPLC were performed on a system equipped with a Büchi pump B-688, Büchi B-684 Fraction collector, UVOLOG-5IIIA UV-Detector, and Büchi columns and precolumns, with the stationary phase silica gel 60 (15-40μm, Qingdao Haiyang).

Plant Material. The roots of *S. inappendiculata* were collected in Yunnan province of China and identified by Prof. Wen-Yan Lian (Institute of Medicinal Plant Development) and Prof. Hong Wang (Menglun Botanical Garden). A voucher specimen (YS-9801) was deposited at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

Extraction and Isolation. The dried ground roots (340 g) of the plant material were extracted with 95% EtOH (4L × 4, 2 h each) under reflux. The EtOH extract (64 g) was chromatographed on flash column (silica gel 60) and eluted with a gradient of cyclohexane:$CHCl_3$:CH_3OH:H_2O (50:50:0:0~0:0:50:50) to give 85 fractions. Fractions 1-5 (1.49 g) were isolated by MPLC (silica gel 60) using $CHCl_3$:CH_3OH (1:0~0:1, gradient) to give 69 parts. Parts 38-43 were purified by Sephadex LH 20 and recrystalization to give **2** (8 mg). Parts 51-62 were purified by Sephadex LH 20 to give compound (**1**) (14 mg). Fractions 6-20 (0.76 g) were further separated by MPLC (silica gel 60), eluted with $CHCl_3$:CH_3OH (1:0~0:1, gradient), to give 43 parts. Parts 29-43 were purified by Sephadex LH 20 and preparative TLC ($CHCl_3$:CH_3COCH_3 9:1) to give compound (**3**) (6 mg).

Securixanthone E (1): yellow needles ($CHCl_3$:CH_3OH 1:1), mp 200°C ($CHCl_3$:CH_3OH 1:1); UV $\lambda_{max}^{CH_3OH}$ nm (log ε) : 239 (4.26), 259 (4.20), 319 (3.15), 384 (3.59) ; + NaOAC: 240 , 260, 321, 385 ; IR (KBr)

ν_{max} cm^{-1}: 3420, 1660, 1610, 1580, 1480; EIMS *m/z*: 272.1 [M^+,95], 257.1 [100], 243.1 [55], 229.1 [80], 128.5 [17], 77.0 [10]; HREIMS *m/z* 272.0670 (calcd for $C_{15}H_{12}O_5$ 272.0685); ^{1}H NMR and ^{13}C NMR, see Table 1.

Securixanthone F (2): yellow needles ($CHCl_3$:CH_3OH 1:1), mp 197°C ($CHCl_3$:CH_3OH 1:1); UV $\lambda_{max}^{CH_3OH}$ nm (log ε) : 239 (4.32), 267 (4.39), 401 (3.62) ; + NaOAC : 225, 267, 404 ; + NaOAC + H_3BO_3 : 226, 267, 403; IR (KBr) ν_{max} cm^{-1}: 3440, 3292, 1640, 1610, 1580, 1495, 1460; EIMS *m/z*: 258.1[M^+,90], 243.1 [52], 229.1 [10], 215.1 [100], 186.1 [8], 65.0 [10]; HREIMS *m/z* 258.0504 (calcd for $C_{14}H_{10}O_5$ 258.0528); ^{1}H NMR and ^{13}C NMR, see Table 1.

Securixanthone G (3): yellow needles ($CHCl_3$:CH_3OH 1:1), mp 162-164°C ($CHCl_3$:CH_3OH 1:1); UV $\lambda_{max}^{CH_3OH}$ nm (log ε): 239 (3.87), 286 (3.51), 359 (3.11) ; + NaOAC : 226, 353 ; + NaOAC + H_3BO_3 : 227, 286, 313, 357; IR (KBr) ν_{max} cm^{-1}: 3400, 1650, 1610, 1580, 1479; HREIMS *m/z* 318.0737 (calcd for $C_{16}H_{14}O_7$ 318.0739); ^{1}H NMR and ^{13}C NMR, see Table 1.

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