Pergamon

0031-9422(95)00277-4

Phytochemistry, Vol. 40, No. 3, pp. 945–947, 1995

0031 9422/95 $9.50 + 0.00

FLAVONOIDS AND A PRENYLATED XANTHONE FROM *CUDRANIA COCHINCHINENSIS* VAR. *GERONTOGEA*

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(*Received* 31 *October* 1994)

Key Word Index—*Cudrania cochinchinensis* var. *gerontogea*; Moraceae; roots; Hwang-jin-quey; methoxylated isoflavone; prenylated xanthone; ^{13}C NMR.

Abstract—A new methoxylated isoflavone, 7,4′-dihydroxy-5,3′,-dimethoxyisoflavone, named gerontosioflavone A, together with 12 known flavonoids, wighteone, genistein, genistein 5-methyl ether, orobol, 6-C-prenylorobol, 6-C-prenylapigenin, 8-C-prenylapigenin, naringenin, 5,7,2′,4′-tetrahydroxyflavanone, artocarpesin, kaempferol and aromadendrin, and a new prenylated xanthone, 4′,5′-dihydro-5,6-dihydroxy-1-methoxy-4′,4′,5′-trimethylfurano (2′,3′: 3,2) xanthone named gerontoxanthone J, together with kaempferol-7-glucoside and kaempferol-3,7-diglucoside, were isolated from root wood and root bark of the Formosan medicinal plant, *Cudrania cochinchinensis* var. *gerontogea*. The structures of the two new compounds were established by 2D NMR techniques.

INTRODUCTION

The roots of *Cudrania cochinchinensis* var. *gerontogea* named 'Hwang-jin-guey' in Taiwan, is used in treatment of neuralgia, rheumatics, hepatitis, contused wounds, etc. [1–3]. In a series of studies on this Taiwan folk medicine, 14 xanthones, including eight novel isoprenylated xanthones, gerontoxanthones A–I, and six known compounds, osajaxanthone, cudraniaxathone, cudraxanthone A, cudraxanthone K, 1,3,7-trihydroxyxanthone and lancerin, were isolated from the root bark of this species [4, 5–8]. From biological tests, we reported that total extracts and fractions from the ethanolic extract of roots of this species also showed significant anti-inflammatory and liver protective effects [6, 7]. Furthermore, ethanolic extracts of the root and the isolated xanthone compounds exhibited anti-lipid peroxidative activities both *in vivo* and *in vitro* [8].

In continuing our search for active constituents of this folk medicine, a new isoflavone, named gerontoisoflavone A, together with 12 known flavonoids were isolated from the ethyl acetate-soluble fraction, the anti-inflammatory and liver protective active fraction [7] of the root wood. Gerontoxanthone J, a new prenylated xanthone, and two flavonol glycosides, kaempferol-7-glucoside and kaempferol-3,7-diglucoside were isolated from the benzene and *n*-butanol fractions of the root bark.

In this paper, we report on the isolation and structural elucidation of the two new compounds, along with the known flavonoids.

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RESULTS AND DISCUSSION

The ethyl acetate-soluble fraction of an ethanolic extract of fresh root wood of *C. cochinchinensis* var. *gerontogea* yielded a new isoflavone, named gerontoisoflavone A (**5**), 7,4′-dihydroxy-5,3′-dimethoxyisoflavone, and 12 known flavonoids, wighteone (**1**), 6-C-prenylapigenin (**2**), 8-C-prenylapigenin (**3**), naringenin (**4**), 6-C-prenylorobol (**6**), genistein 5-methyl ether (**7**), 5,7,2′,4′-tetrahydroxyflavanone (**8**), artocarpesin (**9**), orobol (**10**), genistein (**11**), kaempferol (**12**) and aromadendrin (**13**).

From fresh root bark, a new prenylated xanthone, named gerontoxanthone J (**16**), was isolated from the benzene residue, together with two triterpenoids, butyrospermol acetate (**14**) and butyrospermol (**15**). Two flavonol glycosides, kaempferol-3,7-diglucoside (**17**) and kaempferol-7-glucoside (**19**) were isolated from the *n*-butanol residue.

The known flavonoids and flavonol glycosides were identified by comparison with authentic samples (wighteone, genistein and genistein 5-methyl ether) or from reported spectral data [9–14].

Gerontoisoflavone A (**5**) was assigned the molecular formula $C_{17}H_{14}O_6$ (*m/z* 314). The characteristic colour reaction with Mg + HCl, a typical singlet proton signal at δ8.07 in the ^{1}H NMR spectrum, and the UV spectra were indicative of a 5,7,3′,4′-tetraoxygenated isoflavone chromophore [15]. In addition to the two D_2O-exchangeable proton signals at δ10.66 and 9.00 for two hydroxyl groups, and two signals at δ3.78 (3H, *s*) and 3.73 (3H, *s*) for two methoxyl groups, the ^{1}H NMR spectrum also showed five proton signals at 7.08 (1H, *d*, J = 1.2 Hz), 6.88 (1H, *dd*, J = 8.0 and 2.0 Hz), 6.77 (1H, *d*, J = 8.0 Hz), and 6.38 (2H, *d*, J = 2.4 Hz) for H-2′, H-6′,

H-5′, H-8 and H-6, respectively. Due to the lack of a chelated OH signal near δ13.0 in the ^{1}H NMR spectrum and no shifts on addition of $AlCl_3$ in the UV spectrum, one of the two methoxyl groups at δ3.73 should be located at C-5. Compared with the ^{13}C NMR spectra of the other isolated isoflavones in this species, it was suggested that compound **5** had two possible structures, i.e. 7,4′-dihydroxy-5,3′-dimethoxyisoflavone or 7,3′-dihydroxy-5,4′-dimethoxyisoflavone. The 2D NOESY spectrum (Fig. 1), however, led us to conclude that the structure of **5** was 7,4′-dihydroxy-5,3′-dimethoxy isoflavone. This proposed structure was also confirmed by the ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and HMBC spectra (Fig. 1).

Gerontoxanthone J (**16**) was assigned the molecular formula $C_{19}H_{18}O_6$ by HR-mass spectrometry (MS *m/z* 342.1080). The characteristic colour reaction with Mg + HCl and the UV spectrum were indicative of a 1,3,5,6-tetraoxygenated xanthone chromophore [4, 5]. The ^{1}H NMR spectrum showed the presence of a 2,3-dihydro-2,3,3-trimethylfuran ring (δ1.08, *s*, Me; δ1.36, *s*, Me; δ1.34, *d*, J = 6.6 Hz, Me; δ4.48, *q*, J = 6.6 Hz), three aromatic protons, a pair of *ortho*-coupling protons at δ6.82 (*d*, J = 8.6 Hz, H-7) and δ7.39 (*d*, J = 8.6 Hz, H-8), a signlet proton at δ6.62 (H-4, or H-2), and a methoxyl group at δ4.0. The remaining signals were two singlets at δ10.26 (1H, *br*, ex D_2O) and 9.23 (1H, *br*, ex D_2O) corresponding to two phenolic hydroxyl protons. No bathochromic shift in the UV spectrum atfer adding $AlCl_3$ reagent, and no chelated hydroxyl group signal in ^{1}H NMR spectrum, were observed, indicating that the methoxyl group was on C-1 and the *ortho*-dihydroxyl group on C-5 and C-6. The above evidence led us to conclude that the structure of **16** was 4′,5′-dihydro-5,6-dihydroxy-1-methoxy-4′,4′,5′-trimethylfurano (2′,3′:3,2) xanthone. This proposed structure was also confirmed by ^{13}C NMR, ^{1}H–^{13}C COSY and HMBC spectra (Fig. 2).

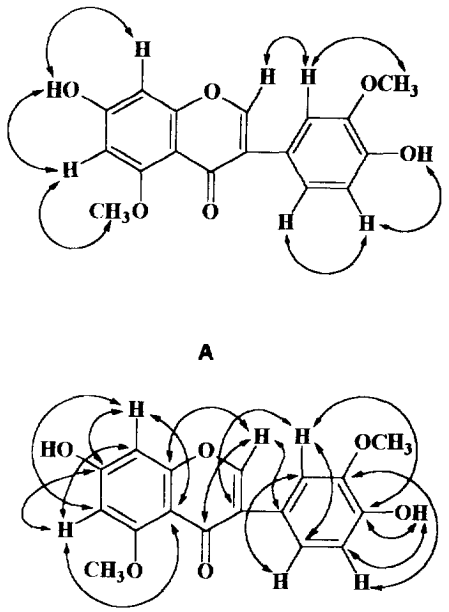

Fig. 1. 2D NOESY (A) and HMBC (B) spectra of compound **5**.

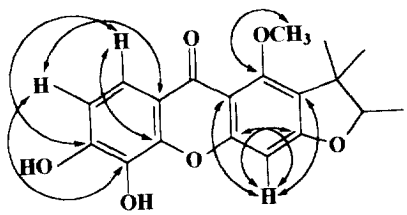

Fig. 2. HMBC spectrum of compound **16**.

EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR (400 and 22.5 MHz, respectively) were recorded with TMS as int. standard. MS were measured at 70 eV. Kieselgel GF-254 and cellulose F (Merck) were used for TLC.

Extraction and separation. Root wood of *C. cochinchinensis* var. *gerontogea* (3.5 kg) was chopped and extracted ×4 with EtOH. The EtOH extract was evapd under red. pres. and extracted with benzene, $CHCl_3$, EtOAc and *n*-BuOH [5]. The EtOAc extract was fractionated sequentially on a silica gel column using $CHCl_3$ and $CHCl_3$–MeOH to give frs A–P. Each fr. was further subjected to repeated CC on silica gel, eluting with a gradient of benzene and EtOAc, followed by polyamide CC with MeOH. These procedures led to the isolation of the following compounds: wighteone (3.5 g, **1**) from fr. B; 6-C-prenylapigenin (25 mg, **2**) and 8-C-prenylapigenin (20 mg, **3**), naringenin (16 mg, **4**) from fr. C; gerontoisoflavone A (38 mg, **5**) from fr. D; 6-C-prenylorobol (56 mg, **6**), genistein 5-methyl ether (22 mg, **7**), and 5,7,2′,4′-tetrahydroxyflavanone (22 mg, **8**) from fr. E; artocarpesin (25 mg, **9**) and orobol (37 mg, **10**) from fr. F; genistein (35 mg, **11**) from fr. G; kaempferol (26 mg, **12**) and aromadendrin (18 mg, **13**) from fr. H.

Fresh root bark (1.5 kg) of *C. cochinchinensis* var. *gerontogea* was extracted with MeOH and the combined MeOH solns evapd under red. pres. The MeOH extract was extracted to give benzene, $CHCl_3$, EtOAc and *n*-BuOH extracts [5]. A portion of the benzene extract was chromatographed on a silica gel column and successively eluted with benzone, benzene–EtOAc and $CHCl_3$–MeOH to give frs A–H. Fr. A (benzene) led to the isolation of butyrospermol acetate (3.68 g, **14**) and butyrospermol (38 mg, **15**). Fr. E (benzene–EtOAc, 4:1–2:1) was subjected to further silica gel CC and eluted with, benzene–EtOAc (6:1–2:1) Fr. C (benzene–EtOAc, 4:1–3:1) was purified by polyamide CC to yield compound **16** (32 mg, frs 19–24, elution with MeOH). The

residue from the *n*-BuOH fr. was sepd by polyamide CC, eluting successively with H_2O and MeOH, leading to the isolation of kaempferol-3,7-diglucoside (16 mg, **17**) from H_2O and kaempferol-7-glucoside (3.4 g, **19**) from 30–80% MeOH.

Gerontoisoflavone A (**5**). Needles (MeOH), mp 283–285°. Brown under UV light, red with (Mg + HCl and brownish green with $FeCl_3$. TLC: $R_f = 0.33$ (silica gel, benzene–EtOAc, 4:1). $C_{17}H_{14}O_6$. EIMS *m/z* (rel. int.): 314 $[M]^+$ (100), 297 $[M - Me]^+$ (13), 285 (11), 268 (15), 148 (9), 133 (11). IR λ_{max}^{KBr} cm^{-1}: 1660 (conj. ketone). UV ν_{max}^{MeOH} nm (log ε): 257 (4.41), 286 (4.13); + $AlCl_3$: 256.6, 285; + NaOAc: 262, 306.8. ^1H NMR (DMSO-d_6): δ10.66 (1H, *br*, OH), 9.0 (1H, *br*, OH), 8.07 (1H, *s*, H-2), 7.08 (1H, *d*, $J = 1.2$ Hz, H-2′), 6.88 (1H, *dd*, $J = 8.0$ and 2.0 Hz, H-6′), 6.77 (1H, *d*, $J = 8.0$ Hz, H-5′), 6.38 (2H, *d*, $J = 2.4$ Hz, H-8 and H-6), 3.78 (3H, *s*, OMe), 3.73 (3H, *s*, OMe). ^{13}C NMR (DMSO-d_6): δ173.9 (C-4), 162.5 (C-7), 161.4 (C-5), 159.2 (C-9), 150.7 (*d*, C-2), 147.2 (C-3′), 146.5 (C-4′), 124.8 (C-3), 123.5 (C-1′), 121.7 (*d*, C-6′), 115.2 (*d*, C-5′), 113.7 (*d*, C-2′), 108.1 (C-10), 96.7 (*d*, C-6), 94.9 (*d*, C-8), 56.0 (OMe), 55.8 (OMe).

Gerontoxanthone J (**16**). Brownish-yellow needles (MeOH), mp 278–280°. $[\alpha]^{25}$°: 0 (MeOH; *c* 0.1). HRMS *m/z*: $C_{19}H_{18}O_6$, found: 342.1080, calcd for [M] 342.1103. TLC: $R_f = 0.33$ (silica gel, benzene–EtOAc, 4:1); $R_f = 0.31$ (cellulose, 15% HOAc). Brown under UV light, red colour with Mg + HCl and dark green with $FeCl_3$. UV λ_{max}^{MeOH} nm (log ε): 213.6 (4.11), 253.6 (4.40), 285.4 (4.01), 310.2 (3.69); + $AlCl_3$: 215, 253.8, 287.8, 311; + $AlCl_3$/HCl: 216.4, 254.2, 287.0, 312.2; + NaOAc: 214.0, 253.4, 285.0, 339.0; + NaOAc/H_3BO_3: 210.4, 218.0, 225.8, 332.0. IR ν_{max}^{KBr} cm^{-1}: 1650 (conj. ketone). EIMS *m/z* (rel. int.): 342 $[M]^+$ (30.8), 327 $[M - Me]^+$ (100), 312 (4.0), 299 (14.7), 283 (4.3), 269 (10.6). ^1H NMR (DMSO-d_6): δ10.26 (1H, *br*, ex. D_2O, 6-OH), 9.23 (1H, *br*, ex, D_2O, 5-OH), 7.39 (1H, *d*, $J = 8.6$ Hz, H-8), 6.82 (2H, *d*, $J = 8.6$ Hz, H-7), 6.62 (1H, *s*, H-4), 4.48 (1H, *m*, H-12), 4.00 (3H, *d*, OMe), 1.08 + 1.36 (3H + 3H, *s* + *s*, 2 × Me). 1.34 (3H, *d*, $J = 6.0$ Hz, Me). ^{13}C NMR (DMSO-d_6): δ173.5 (C-9), 160.7 (C-3), 158.8 (C-1), 157.9 (C-4a), 150.8 (C-6), 145.9 (C-4b), 132.5 (C-5), 118.6 (C-2), 116.2 (*d*, C-8), 115.6 (C-8a), 112.6 (*d*, C-7), 103.5 (C-9a), 92.6 (*d*, C-4), 90.3 (*q*, C-12), 56.3 (OMe), 43.0 (C-11), 25.4 (C-13), 21.0 (C-14), 14.3 (C-15).

Acknowledgements—The authors are deeply indebted to Dr T. H. Wang (President of Chia-Nan Junior College of Pharmacy) for his encouragement and Prof. Satoshi Tahara (Hokkaido University, Japan) for supplying authentic samples of wighteone, genistein, and genistein 5-methyl ether. Thanks also to the National Science Council of the Republic of China for finanical support (NSC-82-0412-B041-009).

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