CONSTITUENTS FROM THE LEAVES OF *LANTANA CAMARA* (IV)

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Eight triterpenoids, betulonic acid (3), betulinic acid (4), oleanolic acid (5), lantadene A (6), lantadene B (7), icterogenin (8), lantanilic acid (9), and ursolic acid (10), three flavonoids, hispidulin (11), pectolinarigenin (12), and pectolinarin (13), as well as β-sitosteryl-3-*O*-β-D-glucoside (2) and a mixture of campesterol (1a), stigmasterol (1b), and β-sitosterol (1c) were isolated from the leaves of the yellow flowering taxa of *Lantana camara* L. The structures of these compounds were established by spectroscopic methods and 2D NMR techniques.

Key words: *Lantana camara* L, Yellow flowering taxa, Triterpenoid, Flavonoid.

INTRODUCTION

Lantana camara L. (Verbenaceae) is a rambling shrub with a number of flower colors viz. red, pink, white, yellow and violet[1]. These plants are cultivated as ornamental or hedge plants in Taiwan[2].

Previously, we reported the phytochemical studies on the stems from the red, pink and yellow flowering taxa of *L. camara* L[3-5]. Herein, we report the isolation of thirteen compounds from the leaves of yellow flowering taxa of this plant, collected at Taichung, Taiwan.

RESULTS AND DISCUSSION

Compounds **1-13** were isolated from the ethyl acetate extract of the leaves of this plant as described in the experimental section. Compound **1** was identified as a mixture of campesterol (**1a**), stigmasterol (**1b**) and β-sitosterol (**1c**) by spectroscopic method and GC analysis[4]. Compound **2** was identified as β-sitosteryl-3-*O*-β-D-glucoside by spectroscopic and chemical methods[4]. In addition, betulonic acid (**3**)[4], betulinic acid (**4**)[3], oleanolic

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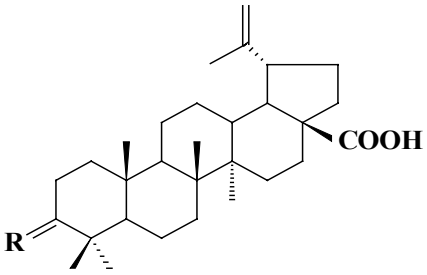
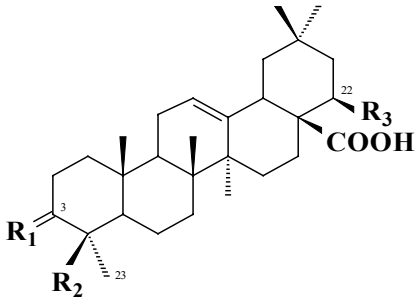
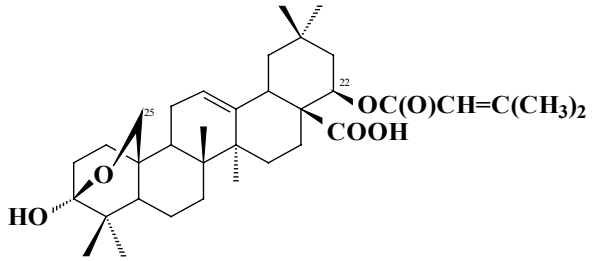


3 : R = O
4 : R = β-OH



5 : R_1 = β-OH R_2 = CH_3 R_3 = H
6 : R_1 = O R_2 = CH_3 R_3 = angeloyl
7 : R_1 = O R_2 = CH_3 R_3 = senecioyl
8 : R_1 = O R_2 = CH_2OH R_3 = angeloyl



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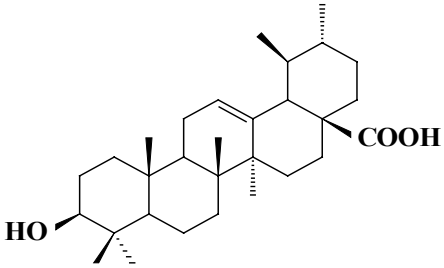

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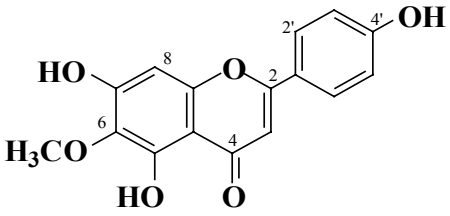
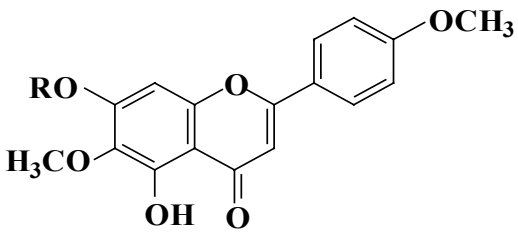
acid (**5**)[3], lantadene A (**6**)[3], lantadene B (**7**)[3], icterogenin (**8**)[4], lantanilic acid (**9**)[5], ursolic acid (**10**)[3], and hispidulin (**11**)[4] were also identified by comparing their spectral data with those in literature.

Compound **12**, obtained as yellow needles, mp: 210-211 °C, gave positive ferric chloride and Mg-HCl tests suggesting the presence of a flavonoid skeleton[6]. It showed an ion peak at *m/z* 315 ($[M + 1]^+$ which was 14 mass units more than hispidulin (**11**) in the FABMS spectrum. The UV spectral behavior of **12** with diagnostic reagents like NaOAc and $AlCl_3$ indicated the presence of the free 5- and 7-hydroxy groups[7]. Comparison of C-6 (δ 131.05) and C-8 (δ 94.05) chemical shifts in the ^{13}C NMR spectrum of **12** with those (C-6, δ 131.11 and C-8, δ 94.05) of **11** showed the presence of 6-methoxy group[4]. The ^1H NMR spectrum showed an A_2X_2 system at δ 6.94 and 7.77 (each 2H, J = 9.0 Hz) due to a 4′-oxygenated B ring[8]. The attachment of methoxyl group to C-4′- was confirmed by the correlation between H-3′ and a methoxyl signal at δ 3.81 and between H-2′ and H-3 in the NOESY spectrum. Furthermore, the HMBC spectrum indicated the correlations of H-3/C-2, C-4, C-1′, H-8/C-6, C-7, C-9, C-10, and 6-OCH_3 (δ 3.86)/C-6. Thus, **12** was deduced as pectolinarigenin and also confirmed by comparison of these data with those in the literature[9].

Compound **13**, obtained as a yellow needle, mp > 300 °C, also gave positive ferric chloride and Mg-HCl tests. It possessed an ion peak at *m/z* 623 ($[M + 1]^+$) in FABMS spectrum. The NMR signals of **13** were similar to





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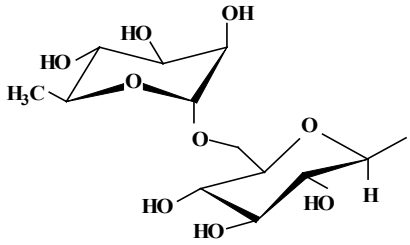
12 : R = H

13 : R =



those of **12**. The only difference was the appearance of two additional glycosyl signals. Two anomeric protons [δ 5.26 (1H, *br s*, rhamnosyl H-1) and 5.63 (1H, *d*, *J* = 7.3 Hz, glucosyl H-1)] together with signals at δ 3.60 (1H, *dd*, *J* = 11.5, 4.9 Hz, glucosyl H_a-6), 3.75 (1H, *dd*, *J* = 11.5, 2.5 Hz, glucosyl H_b-6) and 1.18 (3H, *d*, *J* = 6.3 Hz, rhamnosyl H-6) revealed the rutinosyl moiety. The position of rutinosyl moiety on C-7 was confirmed by the HMBC correlation between glucosyl H-1 (δ 5.63) and C-7 (δ 157.7). Acid hydrolysis of **13** yielded an aglycone pectolinarigenin (**12**), D-glucose and L-rhamnose which were identified by the ^{1}H NMR and thin-layer chromatography. According to the coupling constant, the configuration of D-glucose and L-rhamnose was determined as β- and α-linkage, respectively[10]. The ^{13}C NMR data of **13** also supported the attachment of rutinosyl moiety to the 7-position of pectolinarigenin. Thus, **13** was identified as pectolinarin and also confirmed by comparison these data with those in the literature[11]. This is the first report of the isolation of **12** and **13** from the genus *Lantana.*

EXPERIMENTAL

Melting points were measured on a Yanaco micro melting point apparatus and are uncorrected. IR spectra were recorded on a HITACHI I-2001 infrared spectrometer. EIMS and FABMS spectra were measured by Finnigan MAT 95 XL spectrometer. NMR spectra were recorded on Varian VXR-300 or Unity Inova 600 MHz spectrometers with TMS as an internal standard. Optical rotations were measured on a Perkin Elmer 241 MC digital polarimeter. UV spectra were recorded on a Shimadzu UV 260 ultraviolet spectrometer.

Plant Material

The leaves of *Lantana camara* L. (yellow flower) were collected in the campus of Providence University, Taichung in July 2000. The plant was identified by Mr. Nien-Yung Chiu (Institute of Chinese Pharmaceutical Sciences, China Medical University).

Extraction and Separation

The air-dried leaves (11.9 Kg) of *L. camara* were extracted with MeOH under reflux. The extract was concentrated *in vacuo*. The residue (660 g) was suspended in water and consecutively extracted with ethyl acetate and *n*-butanol. The ethyl acetate fraction (185 g) was chromatographed on a silica gel column using mixtures of $CHCl_3$-MeOH as eluent to give three fractions. Fraction I was purified by repeated column chromatography on silica gel eluting with *n*-hexane-ethyl acetate in order of increasing polarity to afford phytosterols (**1**, 25 mg), betulonic acid (**3**, 70 mg), betulinic acid (**4**, 10 mg), oleanolic acid (**5**, 1510 mg) and ursolic acid (**10**, 15 mg). Fraction II was further chromatographed on a silica gel column with *n*-hexane by increasing amounts of acetone to yield lantadene A (**6**, 20 mg), lantadene B (**7**, 210 mg), icterogenin (**8**, 25 mg) and lantanilic acid (**9**, 5120 mg). Fraction III was rechromatographed on a silica gel column with chloroform and methanol as eluent to give hispidulin (**11**, 780 mg), pectolinarigenin (**12**, 5 mg), pectolinarin (**13**, 25 mg) and β-sitosteryl-3-*O*-β-D-glucoside (**2**, 10 mg).

Pectolinarigenin (12)

Yellow needles ($CHCl_3$/MeOH), mp: 210-211 °C; IR (KBr) ν_{max} cm^{-1}: 3320 (OH), 1660, 1580; UV λ_{max} nm (MeOH) (log ε): 276 (4.10), 333 (4.20), λ_{max} nm (MeOH + NaOMe) (log ε): 276 (4.22), 296 (4.12), 368 (4.02); λ_{max} nm (MeOH + NaOAc) (log ε): 276 (4.27), 297 (4.10), 368 (4.04); λ_{max} nm (MeOH + $AlCl_3$) (log ε): 283 (4.06), 300 (4.11), 354 (4.23); ^{1}H-NMR (600 MHz, $CDCl_3$/CD_3OD): δ 3.81 (3H, *s*, 4′-OCH_3), 3.86 (3H, *s*, 6-OCH_3), 6.47 (1H, *s*, H-3), 6.48 (1H, *s*, H-8), 6.94 (2H, *d, J* = 9.0 Hz, H-3′ and H-5′), 7.77 (2H, *d, J* = 9.0 Hz, H-2′ and H-6′), 13.08 (1H, *br s*, 5-OH); ^{13}C-NMR (150 MHz, $CDCl_3$/CD_3OD): δ 60.5 (*q*, 6-OCH_3), 55.3 (*q*, 4′-OCH_3), 164.2 (*s*, C-2), 103.3 (*d*, C-3), 182.8 (*s*, C-4), 153.1 (*s*, C-5), 131.1 (*s*, C-6), 156.4 (*s*, C-7), 94.1 (*d*, C-8), 152.3 (*s*, C-9), 105.0 (*s*, C-10), 123.3 (*s*, C-1′), 127.9 (*d*, C-2′ and C-6′), 114.3 (*d*, C-3′ and C-5′), 162.5 (*s*, C-4′); FABMS *m/z* (rel. int.%): 315 $[M + 1]^+$ (73), 289 (26), 217 (10), 154 (100), 136 (74).

Pectolinarin (13)

Yellow needles ($CHCl_3$/MeOH); mp > 300 °C; IR (KBr) ν_{max} cm^{-1}: 3390 (OH), 1660, 1610; UV λ_{max} nm (MeOH) (log ε): 276 (4.10), 333 (4.20), λ_{max} nm (MeOH + NaOMe) (log ε): 276 (4.22), 296 (4.12), 368 (4.02); λ_{max} nm (MeOH + NaOAc) (log ε): 276 (4.27), 297 (4.10), 368 (4.04); λ_{max} nm (MeOH + $AlCl_3$) (log ε): 283

(4.06), 300 (4.11), 354 (4.23); ^{1}H-NMR (600 MHz, C_5D_5N): δ 1.18 (3H, *d*, *J* = 6.3 Hz, H-6, Rha), 3.60 (1H, *dd*, *J* = 11.5, 4.9 Hz, H_a-6, Glc), 3.70 (3H, *s*, 4′-OCH_3), 3.75 (1H, *dd*, *J* = 11.5, 2.5 Hz, H_b-6, Glc), 4.04 (3H, *s*, 6-OCH_3), 5.26 (1H, *s*, H-1, Rha), 5.63 (1H, *d*, *J* = 7.3 Hz, H-1, Glc), 6.88 (1H, *s*, H-3), 7.26 (2H, *d, J* = 9.0 Hz, H-3′ and H-5′), 7.32 (1H, *s*, H-8), 8.05 (2H, *d, J* = 9.0 Hz, H-2′ and H-6′), 13.60 (1H, *s*, 5-OH); ^{13}C-NMR (150 MHz, C_5D_5N): δ 55.4 (*q*, 4′-OCH_3), 60.8 (*q*, 6-OCH_3), 164.7 (*s*, C-2), 104.2 (*d*, C-3), 183.1 (*s*, C-4), 154.0 (*s*, C-5), 133.8 (*s*, C-6), 157.7 (*s*, C-7), 95.0 (*d*, C-8), 153.1 (*s*, C-9), 107.1 (*s*, C-10), 123.5 (*s*, C-1′), 128.8 (*d*, C-2′ and C-6′), 115.2 (*d*, C-3′ and C-5′), 163.0 (*s*, C-4′), 102.3 (*d*, C-1, Glc), 74.6 (*d*, C-2, Glc), 78.4 (*d*, C-3, Glc), 71.2 (*d*, C-4, Glc), 77.6 (*d*, C-5, Glc), 67.5 (*t*, C-6, Glc), 102.4 (*d*, C-1, Rha), 72.0 (*d*, C-2, Rha), 72.8 (*d*, C-3, Rha), 74.0 (*d*, C-4, Rha), 69.8 (*d*, C-5, Rha), 18.5 (*q*, C-6, Rha); FABMS *m/z* (rel. int.%): 623 $[M + 1]^+$ (2), 460 (3), 307 (27), 289 (17), 154 (100).

Acid hydrolysis of 13

A solution of **13** (10 mg) in 5% H_2SO_4 (10 mL) was refluxed for 4 hr. The solid, separated after cooling, was recrystallized from MeOH to give yellow needles (3 mg) of an aglycone (**12**), mp 210-211 °C. The aqueous layer was concentrated *in vacuo*. D-glucose and L-rhamnose were identified by TLC on cellulose in pyridine-EtOAc-HOAc-H_2O (5:5:1:3) R_f 0.4 and 0.6, respectively.

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黃花馬纓丹葉部成分之研究

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本研究由黃花馬纓丹 [*Lantana camara* Linn. (yellow taxa)] 葉部分離得到十三個成分，經光譜分析確定其結構分別為 phytosterols (**1**), β-sitosterol-3-O-β-D-glucoside (**2**), betulonic acid (**3**), betulinic acid (**4**), oleanolic acid (**5**), lantadene A (**6**), lantadene B (**7**), icterogenin (**8**), lantanilic acid (**9**), and ursolic acid (**10**), hispidulin (**11**), pectolinarigennin (**12**)及 pectolinarin (**13**)。其中 pectolinarigenin (**12**)及 pectolinarin (**13**)是馬纓丹屬植物文獻上未曾報導的成分。

關鍵詞：黃花馬纓丹，馬鞭草科，三萜類，黃酮類，黃酮類配醣體。

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