Constituents of the Whole Herb of *Clinoponium laxiflorum*

Yueh-Hsiung Kuo*[a] (• • • •), Shu-Mei Lee[b] (• • • •) and Jeng-Shiow Lai[b] (• • • •)
[a]*Department of Chemistry, National Taiwan University, Taipei, Taiwan, R.O.C*
[b]*Department of Applied Chemistry, Providence University, Taichung ,Taiwan, R.O.C*

The isolation and identification of twenty-two components (including one new compound) from the whole herb of *Clinoponium laxiflorum* (Hay) Matsum (Labiatae) are described. Their structures were determined on the basis of spectral and chemical transformation. One new compound is methyl rosmarinate. The other twenty-one compounds include three steroids (α-spinasterol, α-spinasteryl-3-*O*-β-D-glucopyranoside, and β-sitosteryl-3-*O*-β-glucopyranoside), three triterpenes (oleanolic acid, ursolic acid, and betulinic acid), nine flavonoids (didymin, apigenin-7-*O*-β-glucopyranoside, luteolin-7-*O*-β-glucopyranoside, isosakuranetin, narigenin, apigenin, luteolin, narirutin, and hesperidin), three lignolic acids (rosmarinic acid, 3-(3,4-dihydroxyphenyl)lactic acid, and caffeic acid), and three phenols (4-hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde, and 3,4-dihydroxybenzoic acid).

INTRODUCTION

Clinopodium gracile (Benth) O. Ktze, *C. laxiforum* (Hay.) Matsum., and *C. umbrosum* (Bieb.) C. Koch are only three species of *Clinopodium* in Taiwan. The latter species has been used in folk medicine as an antibiotic and for treatment of inflammation and bleeding.[1,2] Only one species of *Clinopodium*, *C. chinensis*,[3] has been investigated for its chemical constituents, and five components, ursolic acid, isosakuranetin, apigenin, didymin, and hesperidin have been reported. We have investigated the constituents of the whole herb of *C. umbrosum*, and fifteen components including five steroids, four triterpenes, four flavonoids, and two lignolic acids have been reported.[5] In connection with our interest in flavonoids, we investigated the chemical constituents of the whole herb of *C. laxiflorum*. One new compounds, methyl rosmarinate, together with twenty-one known compounds were isolated and purified. In this paper, we describe the structural elucidation of isolated components.

RESULTS AND DISCUSSION

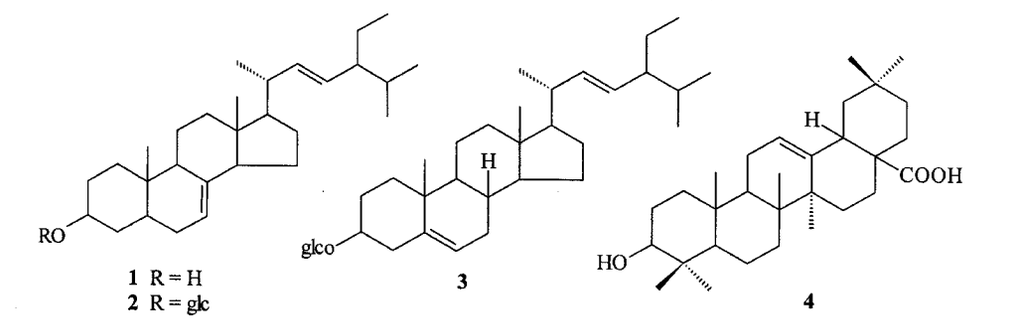
The ethanol extract of the whole herb of *C. laxflorum* was partitioned with ethyl acetate and water. The aqueous layer was subsequently extracted with 1-butanol. After it was purified on silica gel and Sephadex LH-20, steroids [α-spinasterol (**1**),[5] α-spinasteryl-3-*O*-β-D-glucopyranoside (**2**),[5] and β-sitosteryl-3-*O*-β-glucopyranoside (**3**)[6]], triterpenes [oleanolic acid (**4**),[7] ursolic acid (**5**),[6] and betulinic acid (**6**)[8]], flavonoids [isosakuranetin (**7**),[9] didymin (**8**),[10] narigenin (**9**),[11] apigenin (**10**),[12] luteolin (**11**)[13]], lignolic acids [rosmarinic acid (**12**),[5] methyl rosmarinate (**13**), and caffeic acid (**14**)[14]], and phenols [4-hydroxybenzaldehyde (**15**),[14] 3,4-dihydroxybenzaldehyde (**16**),[15] and 3,4-dihydroxybenzoic acid (**17**)] were isolated from the ethyl acetate soluble fraction. The *n*-BuOH layer gave flavonoids [apigenin-7-*O*-β-glucopyranoside (**18**),[17] luteolin-7-*O*-β-glucopyranoside (**19**),[5] hesperidin (**20**),[18] and apigenin-7-*O*-β-methyl glucuronate (**21**)[5]], and lignolic acid [3-(3,4-dihydroxyphenyl)lactic acid (**22**)[5]] after repeated chromatography on Diaion HP-20 and Sephadex LH-20.

Methyl rosmarinate (**13**),[19] reported in a previous communication, was elucidated in detail as follows. Compound **13** is a dilignol based on its molecular formula $C_{19}H_{18}O_8$ which was discerned from its elemental analysis and ^{13}C NMR data. It showed the presence of a hydroxyl group (3397 cm^{-1}), two ester groups (1727 and 1690 cm^{-1}), and a phenyl group (1605 and 1521 cm^{-1})from its IR spectrum. The ^1H NMR spectrum showed two groups of 1,3,4-trisubstituted pheny protons with an ABX-system pattern. The ^{13}C NMR spectrum also showed four oxygenated phenyl carbons at δ 146.1, 146.7, 147.9, and 149.7; meanwhile the signals for two esters (δ 168.3 and 172.2), for conjugated olefin (δ 145.3 and 117.5), for methyl ester (δ 52.6), and for a carbon bearing ester group (δ 74.6) all appeared. An ABX system signals at δ 3.00 (1H, dd, J = 14.1, 7.2 Hz), 3.03 (1H, dd, J = 14.1, 5.4 Hz), and 5.19 (1H, dd, J = 7.2 and 5.4 Hz) were also presented in ^1H NMR spectrum. The UV absorption bands (λ_{max} 287 and 326 nm) and ^1H NMR signals for olefinic protons at δ 6.25 and 7.54 (each 1H, d, J =

Dedicated to the memory of the late Professor Ta-shue Chou

15.9 Hz) revealed it contained a caffeate moiety. The methylation of **13** with potassium carbonate and iodomethane in refluxing acetone yielded a pentamethyl derivative **23** [amorphous, δ 3.68, 3.78, 3.79, 3.82, and 3.84 (each 3H, s)]. A tetraphenolic acetate **24** [amorphous; υ_{max} 1765 and 1725 cm^{-1}; δ 2.24, 2.24, 2.27, and 2.28 (each 3H, s)] was obtained from **13** by acetylation. Methyl rosmarinate (**13**) gave methyl dihydrorosmarinate (**25**) [amorphous; υ_{max} 1725 cm^{-1}; δ 2.53 and 2.64 (each 2H, t, J = 7.0Hz)] by catalytic hydrogenation with 10% Pd-C as catalyst in methanol solution. When **12** was allowed to react with diazomethane in methanol for 10 min at room temperature, it readily afforded **13**. From the above results, the structure of **13** can be assigned as methyl rosmarinate.

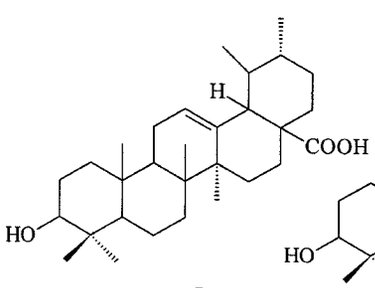
Three steroids all gave a positive Liebermann-Burchurd test. α-Spinasterol (**1**),[5] α-spinasteryl-3-*O*-β-D-glucopyranoside (**2**),[5] and β-sitosteryl-3-*O*-β-glucopyranoside (**3**)[6] were identified by direct comparison with authentic samples. Three triterpenes, oleanolic acid (**4**),[7] ursolic acid (**5**),[6] and betulinic acid (**6**)[8] were also isolated from *Clinopodium umbrosum*,[5] the same genus of this plant. Compounds **7, 8, 9, 10, 11, 18, 19, 20,** and **21** are all flavanoids due to their



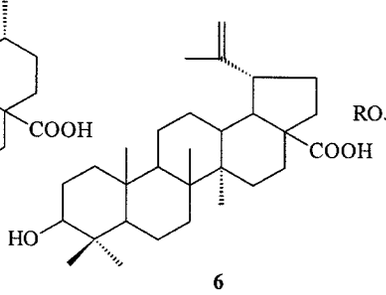
1 R = H
2 R = glc

3

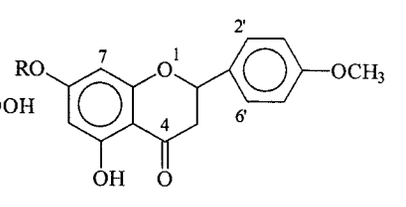
4



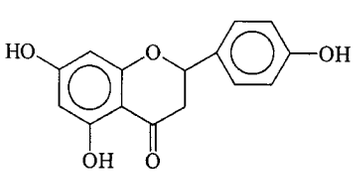
5



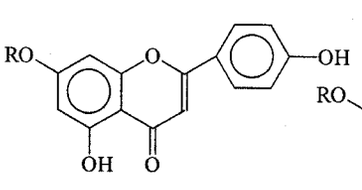
6



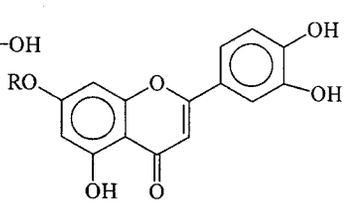
7 R=H
8 R=Rha-(1→6)-glc



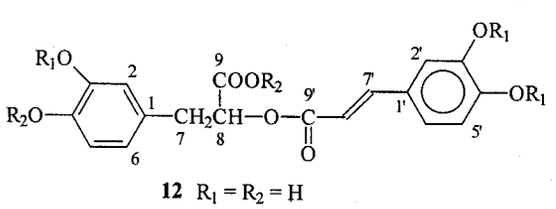
9



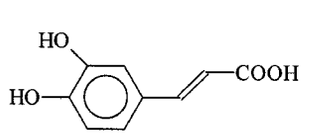
10 R=H
18 R=glc
21 R=



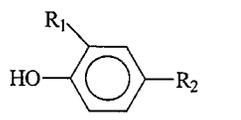
11 R=H
19 R=glc



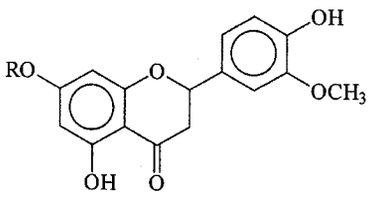
12 $R_1 = R_2 = H$
13 $R_1 = H$, $R_2 = Me$
23 $R_1 = R_2 = Me$
24 $R_1 = Ac$, $R_2 = Me$



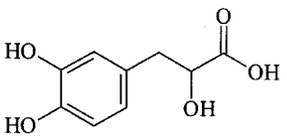
14



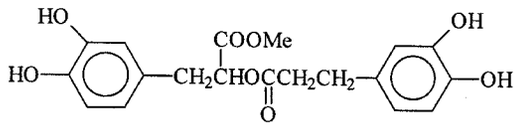
15 R_1 = H, R_2 = CHO
16 R_1 = OH, R_2 = CHO
17 R_1 = OH, R_2 = COOH



20 R=Rha-(1→6)-glc



22



25

postitive Mg-HCl color test. A UV absorption (λ_{max} 290, 328 nm), ABX system signals, an A_2X_2 system of B ring phenyl protons, a phenolic methyl signal, two *meta* –phenyl protons signals as well as a chelated OH group at δ 12.01 indicated compound **7** is an isosakuranetin.[9] Compound **8** exhibited the NMR signals similar to those in compound **7** in addition to two glycosyl units signals. Two anomeric protons [δ 4.69 (1H, s, rhamnosyl H-1) and 4.93 (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, 2.7 Hz, glucosyl H_b-6), and 1.18 (3H, d, J = 6.3 Hz, rhamnosyl H-6) revealed the rutinoside moiety. Therefore, compound **8** was assigned as 5-hydroxy-4'-methoxyflavanone-7-*O*-rutinoside (didymin).[10] Compound **9** has similar ^{1}H NMR signals as **7;** the only difference is a hydroxyl group at C-4' instead of a methoxyl group in **7**. Comparison of their ^{1}H NMR data reported in the literature[11] identified it as naringenin. Compound **10** is a flavone derivative, which showed two *meta*-phenyl protons at δ 6.21 and 6.51 (each 1H, d, J = 1.8 Hz), 6.80 (1H, s, H-3), and A_2X_2 system of B-ring protons. It was assigned as apigenin by comparison of their physical data with those in the literature.[12] Compounds **11**,[13] **19**,[5] and **21**[5] were identified by direct comparison with authentic samples. Compound **18** showed similar ^{1}H NMR data as compound **10** except for an additional glucoside moiety, which was releaved by ^{13}C NMR data. The lowest signal at δ 12.95 (s, C-5 OH) and no shift of band II in UV spectrum as the addition of NaOAc indicated the glucoside moiety is located at C-7. Therefore, **18** was assigned as apigenin-7-*O*-β-glucopyranoside.[17] Compound **20** has a rutinoside moiety as in compound **7**, which was discerned from its ^{13}C NMR data and ^{1}H NMR signals at δ 1.08 (3H, d, J = 6.0 Hz), 4.67 (1H, br s, H-1 of rhamnosyl), 5.35 (1H, d, J = 6.8 Hz, H-1 of glucosyl). It shows the characteristic signal of flavanone ABX system signals: two higher fields of phenyl protons at δ 6.29 (2H, s). No bathochromic shift effect in its UV spectrum as the addition of NaOAc indicates that C-7 OH is not free. Three B-ring phenyl proton signals condensed between δ 6.77~6.84. Comparison of ^{13}C NMR data with that reported in the literature,[18] identified it as hesperidin. The remaining three lignolic acids [rosmarinic acid (**12**)[5] and caffeic acid (**14**),[14] and 3-(3,4-dihydroxyphenyl)lactic acid (**22**)[5]] and three phenols [4-hydroxybenzaldehyde (**15**),[14] 3,4-dihydroxybenzaldehyde (**16**),[15] and 3,4-dihydroxybenzoic acid (**17**)[16]] were all identified by authentic samples.

The above twenty-two compounds contained five different skeletons: three steroids (**1, 2,** and **3**), three triterpenoids (**4, 5,** and **6**), nine flavonoids (**7, 8, 9, 10, 11, 18, 19, 20**, and **21**), four lignoids (**12, 13, 14**, and **22**), and three phenylmethanoids (**15, 16,** and **17**). Flavonoid is a major product in this plant.

EXPERIMENTAL SECTION

Melting points (Yanagimoto micro melting-point apparatus) are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter at room temperature. IR spectra were recorded on a JASCO A-102 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 300 instrument using tetramethylsilane as internal standard. Chemical shifts are given in δ values/ppm and coupling constants (J) are given in hertz (Hz). Electron-impact mass spectrum (EI-MS) were measured on a JEOLJMS-100 spectrometer.

EXTRACTION AND ISOLATION

The air dried whole herb of *C .laxiflooum* (3.5 Kg) was extracted with 95% ethanol (100 L) three times (12 h each time) at 80-85 °C. The combined extracts were evaporated under reduced pressure to give a residue which was dissolved in 500 mL of water and then extracted with ethyl acetate (500 mL × 3). The aqueous layer was subsequently shaken with 1-butanol (500 mL × 3) and gave butanol and water layers. EtOAc and BuOH layers were evaporated to yield residues of 120 g and 100 g, respectively. After repeated purification on silica gel, Diaion HP-20, and Sephadex LH-20, α-spinasterol (**1**, 1.0 g), α-spinasteryl-3-*O*-β-D-glucopyranoside (**2**, 3.1 g), β-sitosteryl-3-*O*-β-glucopyranoside (**3**, 15 mg), oleanolic acid (**4**, 1.8 g), ursolic acid (**5**, 42 mg), betulinic acid (**6**, 12 mg), isosakuranetin (**7**, 20 mg), didymin (**8**, 20 mg), narigenin (**9**, 19 mg) apigenin (**10**, 50 mg), luteolin (**11**, 45 mg), rosmarinic acid (**12**, 3.5 g), methyl rosmarinate (**13**, 30 mg), caffeic acid (**14**, 2.0 g), 4-hydroxybenzaldehyde (**15**, 25 mg), 3,4-dihydroxybenzaldehyde (**16**, 25 mg), 3,4-dihydroxybenzoic acid (**17**, 23 mg) came from the EtOAc layer, and apigenin-7-*O*-β-glucopyranoside (**18**, 3.0 g), luteolin-7-*O*-β-glucopyranoside (**19**, 3.4 g), hesperidin (**20**, 4 mg), apigenin-7-*O*-β-methyl glucuronate (**21**, 30 mg), and 3-(3,4-dihydroxyphenyl)lactic acid (**22**, 20 mg) from the 1-butanol layer. The eluting order of EtOAc layer by SiO_2 chromatography was shown as follows: **1** (20% $CHCl_3$ in hexane), **4**, **5**, **6**, **7**, **15** (30% $CHCl_3$ in hexane), **9**, **10**, **11** (50% $CHCl_3$ in hexane), **17**, **14** (60% $CHCl_3$ in hexane), **2**, **3** (80% $CHCl_3$ in hexane), **8**, **12**, **13** (50% $CHCl_3$ in MeOH, then separated by Sephadex LH-20). The BuOH layer was separated by Diaion HP-20 first (MeOH:H_2O = 1:1), and then rechromatographed on Sephadex LH-20. Compounds **1, 2, 3, 4, 5, 6, 11, 12, 14, 15, 16, 17, 19, 21**, and **22** are identified with authentic samples directly. The physical data of other compounds are shown as follows.

Isosakuranetin (7)

Mp 193-194.5 °C (from acetone); IR (KBr) υ_{max}: 3154, 1630, 1595, 1492, 1299, 1251, 832, 807, 723 cm^{-1}; UV λ_{max} (MeOH): 290, 328 nm; EI-MS *m/z* (%): 286 (M^+, 70), 134 (100), 121 (74); ^1H NMR ($CDCl_3$) δ 2.76 (1H, dd, *J* = 17.1, 3.1 Hz, H_a-3), 3.07 (1H, dd, *J* = 17.1, 12.9 Hz, H_b-3), 3.81 (3H, s, 4'-OCH_3), 5.34 (1H, dd, *J* = 12.9, 3.1 Hz, H-2), 5.96 and 5.28 (each 1H, d, *J* = 2.0 Hz, H-6, -8), 6.93 and 7.35 (each 2H, d, *J* = 8.7 Hz, H-3', -5'; H-2', 6').

Didymin (8)

Mp 213-214 °C (from MeOH); IR (KBr) υ_{max}: 3474, 1641, 1605, 1515, 1155, 1135, 960 cm^{-1}; UV λ_{max} (MeOH): 285, 330 nm; FABMS (negative) *m/z*: 593 $(M-H)^-$, ^1H NMR (CD_3OD) δ 1.18 (3H, d, *J* = 6.3 Hz, rhamnosyl H-6), 2.79 (1H, dd, *J* = 17.2, 3.2 Hz, H_a-3), 3.19 (1H, dd, *J* = 17.2, 12.6 Hz, H_b-3), 3.60 (1H, dd, *J* = 11.5, 4.9 Hz, glucosyl H_a-6), 3.75 (1H, dd, *J* = 11.5, 2.7 Hz, glucosyl H_b-6), 3.80 (3H, s, OCH_3), 4.69 (1H, s, rhamosyl H-1), 4.93 (1H, d, *J* = 7.3 Hz, glucosyl H-1), 5.46 (1H, d, *J* = 12.6, 3.2 Hz, H-2), 6.18, 6.20 (each 1H, d, *J* = 2.3 Hz, H-6, -8), 6.95 and 7.43 (each 2H, dd, *J* = 8.8 Hz, H-3', -5'; H-2', 6'). ^{13}C NMR (DMSO-D_6) δ 78.6 (C-2), 42.2 (C-3), 197.3 (C-4), 163.5 (C-5), 96.7 (C-7), 95.8 (C-8), 162.8 (C-9), 103.6 (C-10), 130.6 (C-11), 128.7 (C-2', -6'), 114.2 (C-3', 5'), 159.8 (C-4'), 99.7 (C-1''), 78.6 (C-2''), 76.5 (C-3''), 71.0 (C-4''), 75.9 (C-5''), 66.3 (C-6''), 100.8 (C-1'''), 71.0 (C-2'''), 69.9 (C-3'''), 72.4 (C-4'''), 68.6 (C-5'''), 18.1 (C-6''''), 55.4 (OCH_3). **Heptaacetate** of **8** (acetylation with Ac_2O and pyridine at 50 °C for 24 h): mp 115-116 °C; IR υ_{max} cm^{-1}: 1753, 1682, 1614, 1568, 1513, 1368, 1217, 1061 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.12 (3H, d, *J* = 6.2 Hz), 1.92, 2.00, 2.00, 2.00, 2.04, 2.06, 2.35 (each 3H, s, -OAc), 2.70 (1H, dd, *J* = 16.7, 2.9 Hz), 3.00 (1H, *J* = 16.7, 13.0 Hz), 3.61 (1H, dd, *J* = 11.5, 2.8 Hz, H_a-6''), 3.80 (3H, s, -OCH_3), 3.82 (3H, m, H-5'', H-5''', H_b-6''), 4.66 (1H, s, H-1'''), 4.95-5.45 (7H, m), 6.28, 6.45 (each 1H, d, *J* = 2.3 Hz), 6.92 and 7.34 (each 2H, d, *J* = 8.7 Hz).

Naringenin (9)

Mp 249-252 °C (from MeOH); IR (KBr) υ_{max}: 3279, 1629, 1599, 1310, 1247, 1179, 1156, 1083, 831 cm^{-1}; UV λ_{max} (MeOH): 289, 326 nm; EI-MS *m/z* (%): 272 (M^+, 100), 179 (31), 153 (93), 120 (73), 107 (24), 91 (27); ^1H NMR (CD_3OD) δ 2.68 (1H, dd, *J* = 17.0, 3.0 Hz), 3.10 (1H, dd, *J* = 17.0, 12.8 Hz), 5.32 (1H, dd, *J* = 12.8, 3.0 Hz), 5.87, 5.89 (each 1H, d, *J* = 1.2 Hz), 6.80 and 7.30 (each 2H, d, *J* = 8.5 Hz).

Apigenin (10)

Mp > 300 °C (from MeOH); IR (KBr) υ_{max}: 3300, 1646, 1603, 1497, 1355, 1240, 1180, 830 cm^{-1}; UV λ_{max} (MeOH): 267, 296 (sh), 336 nm; EI-MS *m/z* (%): 270 (M^+, 100), 242 (3), 153 (11), 152 (4); ^1H NMR (DMSO-D_6) δ 6.21 and 6.51 (each 1H, d, *J* = 1.8 Hz), 6.80 (1H, s), 6.95, 7.94 (each 2H, d, *J* = 8.7 Hz), and 12.98 (1H, s, 5-OH).

Methyl rosmarinate (13)

Amorphous; $[\alpha]_D^{18}$ +138° (*c* 0.6, MeOH); IR (KBr) υ_{max}: 3397, 1727, 1690, 1605, 1521, 1363, 1282, 1160, 1114, 1073, 979 cm^{-1}; UV λ_{max} (MeOH): 287, 326 nm; ^1H NMR (CD_3OD) δ 3.00 (1H, dd, *J* = 14.1, 7.2 Hz, H_a-7), 3.03 (1H, dd, *J* = 14.1, 5.4 Hz), 3.68 (3H, s, -OCH_3), 5.19 (1H, dd, *J* = 7.2, 5.4 Hz, H-8), 6.25 (1H, d, *J* = 15.9 Hz, H-8'), 6.56 (1H, dd, *J* = 8.0, 1.9 Hz, H-6), 6.69 (1H, d, *J* = 8.0 Hz, H-5), 6.73 (1H, d, *J*

= 1.9 Hz, H-2), 6.78 (1H, d, J = 8.2 Hz, H-5') , 6.94 (1H, dd, J = 8.2, 1.9 Hz, H-6') , 7.04 (1H, d, J = 1.9 Hz, H-2') , 7.54 (1H, d, J = 15.9 Hz, H-7'); ^{13}C NMR (CD_3OD) δ 127.5 (C-1), 115.2 (C-2), 146.1 (C-3), 146.7 (C-4), 116.5 (C-5), 121.8 (C-6), 38.8 (C-7), 74.6 (C-8), 172.2 (C-9), 28.7 (C-1') , 114.1 (C-2'), 147.9 (C-3'), 149.7 (C-4'), 116.3 (C-5'), 123.1 (C-6') , 145.3 (C-7'), 117.5 (C-8'), 168.3 (C-9'), 52.6 ($COOCH_3$). Anal. Calcd for $C_{19}H_{18}O_8$; C, 58.46; H, 4.65. Found: C, 58.37; H, 4.60.

Apigenin-7-*O*-β-glucopyranoside (18)

mp: 216-218 °C (from MeOH); IR (KBr) υ_{max}: 3400, 1654, 1620, 1605, 1510, 1250, 1200, 1095, 865 cm^{-1}; UV λ_{max} (MeOH): 266, 333 nm; +$AlCl_3$ 278, 300, 350, 391 nm; +NaOAc 266, 355, 390 nm; FABMS (negative) *m/z*: 431 $(M-1)^-$; ^{1}H NMR (DMSO-d_6) δ 3.71 (1H, m, H-5''), 5.06 (1H, d, J = 6.9 Hz, H-1''), 6.44, 6.82 (each 1H, d, J = 2.0 Hz, H-6, -8), 6.88 (1H, s, H-3), 6.93, 7.95 (each 2H, d, J = 8.7 Hz, H-3', -5'; H-2', -6'), 10.39 and 12.95 (each 1H, s, 2x-OH); ^{13}C NMR (DMSO-d_6) δ 164.4 (C-2), 103.2 (C-3), 182.1 (C-4), 161.5 (C-5), 99.7 (C-6), 163.1 (C-7), 95.0 (C-8), 157.1 (C-9), 105.5 (C-10), 121.2 (C-11), 128.7 (C-2'), 116.1 (C-3'), 161.2 (C-4'), 116.1 (C-5'), 128.7 (C-6'), 100.2 (C-1''), 73.3 (C-2''), 76.6 (C-3''), 69.8 (C-4''), 77.3 (C-5''), 60.8 (C-6'').

Hesperidin (20)

Mp: 256-261 °C (from MeOH); IR (KBr) υ_{max}: 3473, 1646, 1605, 1516, 1277, 1204, 1068, 816 cm^{-1}; UV λ_{max} (MeOH): 283, 326 nm; +$AlCl_3$ 308, 383 nm; + $AlCl_3$+HCl 306, 379 nm ; +NaOAc 283, 327 nm; FABMS (negative): *m/z*: 609 $(M-H)^-$; ^{1}H NMR (CD_3OD) δ 1.08 (3H, d, J = 6.0 Hz, rhamosyl H-6), 2.69 (1H, dd, J = 18.0, 5.0 Hz, H_a-3), 2.99 (1H, dd, J = 18.0, 14.0 Hz, H_b-3), 4.59 (1H, brs, rhamnosyl H-1), 4.95 (1H, d, J = 7.8 Hz, glucosyl H-1), 5.35 (1H, dd, J = 14.0, 5.0 Hz), 6.29 (2H, s, H-6, -8), 6.77-6.84 (3H, m, H-2', -5', -6'); ^{13}C NMR (CD_3OD) δ 78.9 (C-2), 42.0 (C-3), 197.3 (C-4), 163.8 (C-5), 96.9 (C-6), 165.4 (C-7), 95.9 (C-8), 162.8 (C-9), 103.8 (C-10), 131.4 (C-1'), 114.4 (C-2'), 148.2 (C-3'), 146.7 (C-4'), 112.4 (C-5'), 118.3 (C-6'), 99.7 (C-1''), 73.8 (C-2''), 76.6 (C-3''), 71.0 (C-4''), 75.8 (C-5''), 66.4 (C-6''), 100.9 (C-1'''), 70.6 (C-2'''), 69.9 (C-3'''), 72.4 (C-4'''), 68.8 (C-5'''), 18.5 (C-6'''), and 56.0 (OCH_3).

Methylation of 12

A mixture of **12** (20 mg), MeI (5 mL), K_2CO_3 (150 mg) and 15 mL of acetone was stirred 8 h under reflux. After solvent was evaporated, 15 mL of H_2O was poured onto the residue, and the mixture was extracted with EtOAc (10 mL × 3). The extract was purified by silica gel to yield **23** (24 mg) [amorphous; IR (KBr) υ_{max}: 1738, 1708, 1627, 1594, 1262, 1156 cm^{-1}; EIMS *m/z* (%): 430 (M^+, 6), 222 (100), 208 (70), 191 (35), 151 (20); ^{1}H NMR ($CDCl_3$) δ 3.12 (2H, m, H-7), 3.68, 3.78, 3.79, 3.82, 3.84 (each 3H, s, -OCH_3), 5.28 (1H, dd, J = 7.1, 5.3 Hz, H-8), 6.28, 7.56 (each 1H, d, J = 16.0 Hz, H-8', -7'), 6.72 (1H, s, H-2), 6.74, 6.76 (each 1H, d, J = 8.6 Hz, H-5, -6), 6.80 (1H, d, J = 8.2 Hz, H-5'), 6.98 (1H, d, J = 1.8 Hz, H-2'), 7.01 (1H, dd, J = 8.2, 1.8 Hz, H-6'); ^{13}C NMR ($CDCl_3$) δ 128.3 (C-1), 110.9 (C-2), 148.4 (C-3), 148.4 (C-4), 112.5 (C-5), 121.4 (C-6), 37.1 (C-7), 72.9 (C-8), 165.2 (C-9), 127.1 (C-1'), 109.6 (C-2'), 149.2 (C-3'), 151.3 (C-4'), 111.2 (C-5'), 122.8 (C-6'), 145.9 (C-7'), 114.5 (C-8'), 170.3 (C-9'), 52.2 (-$COOCH_3$), 55.8 (4 × Ar-OCH_3)].

Acetylation of 12

Compound **12** (6 mg) was allowed to react with Ac_2O (0.5 mL) and pyridine (0.5 mL) at room temperature overnight. The usual work-up gave **24** (7 mg) [Amorphous; IR (KBr) υ_{max}: 1765, 1725, 1633, 1605, 1499, 1204, 1114, 1044, 1014, 901, 835, 797 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.24, 2.24, 2.27, 2.28 (each 3H, s, -OAc), 3.15 (1H, dd, J = 14.0, 7.5 Hz), 3.17 (1H, dd, J = 14.0, 5.4 Hz), 3.72 (3H, s, -OCH_3), 6.39, 7.30 (each 1H, d, J = 16.0 Hz), 7.07 (1H, s, H-2), 7.10, 7.12 (each 1H, d, J = 8.3 Hz, H-5, -6), 7.20 (1H, d, J = 8.4 Hz, H-5'), 7.36 (1H, d, J = 2.1 Hz, H-2'), 7.39 (1H, dd, J = 8.4, 2.1 Hz, H-6')].

Hydrogenation of 12

Compound **12** (5 mg) in 5 mL of MeOH was hydrogenated in the pressence of 10% Pd-C (5 mg). After 6 h, the catalyst was removed by filtration and washed several times with MeOH. The product yielded **25** (4 mg) [Amorphous; IR (KBr) υ_{max}: 3390, 1725, 1603, 1520, 1363, 1283, 1113, 1067 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.53 (2H, t, J = 7.0 Hz, H-7'), 2.64 (2H, t, J = 7.0 Hz, H-8'), 2.86 (1H, dd, J = 14.2, 7.2 Hz, H_a-7), 2.92 (1H, dd, J = 14.2, 5.4 Hz, H_b-7), 3.61 (3H, s, -OCH_3), 5.12 (1H, t, J = 7.2, 5.4 Hz, H-8), 6.38 (1H, d, J = 7.5 Hz, H-2'), 6.46 (1H, d, J = 7.5 Hz, H-2), 6.60-6.75 (4H, m, H-5, -5'; -6, -6')].

Partial methylation of 12

Excess of diazomethane in diethyl ether was added to a solution of rosmarinic acid (**12**) (5 mg) in methanol (3 mL). After 10 min, the solvent and excess of diazomethane was removed under the reduced pressure. The residue was identified with methyl rosmarinate acid (**13**).

ACKNOWLEDGMENT

We thank the National Science Council of the R.O.C. for financial support.

Received October 30, 1999.

Key Words

Clinopodium laxiflorum; Methyl rosmarinate; Steroid; Triterpene; Flavonoid; Lignolic acid; Phenol.

REFERENCES

1. Ministry of Health of PROC Pharmacopedia Dispensing Information, ROC Pharmacopedia Shin Hua, Beijing, China, **1990**, p. 294.
2. Chiang Su New Medical College, Dictionary of Chinese Crude Drugs Shanghai Scientific Technological Publisher; Hong Kong, **1977**, p. 484.
3. Kong, D.Y.; Dai, J. R; Shi, D.W. *Zhongcaoyao* **1985**, *16*, 518; C.A. (104), 65944W.
4. Dai, J. R.; Shi, D. W.; Zhang, H. Q. *Zhongcaoyao* **1983**, *14*, 193; C.A. (99), 85088b.
5. Lee, S. M.; Lai, J. S.; Kuo, Y. H. *J. Chin. Chem. Soc.* **1993**, *40*, 87.
6. Kuo, Y. H.; Yeh, M. H. *J. Chin. Chem. Soc.* **1997**, *44*, 379.
7. Kuo, Y. H.; Chen; Z. S. ; Lin, Y. L. *Chem. Pharm. Bull.* **1996**, *44*, 429.
8. Kuo, Y. H.; Li, Y. C., *J. Chin. Chem. Soc.* **1997**, *44*, 321.
9. Bohlmann, F.; Zdero, C. *Phytochemistry* **1978**, *17*, 1155.
10. Kamiya, S.; Esaki, S.; Korishi, F. *Agric. Biol. Chem.* **1979**, *43*, 1529.
11. Bohlmann, F.; Suwita, A. *Phytochemistry* **1979**, *18*, 885.
12. Morita, N.; Arisawa, M.; Yoshikawa, A. *Yakugaku Zasshi* **1976**, *96*, 1180.
13. Lin, Y. L.; Ou, J. C.; Chen, C. F.; Kuo, Y. H. *J. Chin. Chem. Soc.* **1991**, *38*, 619.
14. Camparison with an authentic sample which was commercially available.
15. Chen, Z. S.; Lai, J. S.; Kuo, Y. H. *J. Chin. Chem. Soc.* **1991**, *38*, 393.
16. Lin, Y. L.; Tsai, W. J .; Chen, I. S.; Kuo, Y. H. *J .Chin. Chem. Soc.* **1998**, *45*, 213.
17. Hottori, S.; Matsuola, H. *Acta Phytochem Jpn.* **1949**, *15*, 233.
18. Horowitz, R. M.; Gentili, B. *Tetrahedron* **1963**, *19*, 773.
19. Lee, S. M.; Lai, J. S.; Kuo, Y. H. *Chem.Express* **1992**, *7*, 897.
20. Mabry, T. J.; Markham, K. R.; Thomas, M. B. *The Systematic Identification of Flavonoids*; Springer-Verlag: Berlin, **1970**, Chap. V, VIII.