[Chem. Pharm. Bull. 29(1) 254—256 (1981)]

Studies on a Novel *p*-Coumaroyl Glucoside of Apigenin and on Other Flavonoids isolated from Patchouli (Labiatae)

HIDEJI ITOKAWA,* KEIICHI SUTO, and KOICHI TAKEYA

Tokyo College of Pharmacy,[1] 1432-1 Horinouchi, Hachioji, Tokyo, 192-03 Japan

(Received August 8, 1980)

Pachypodol, ombuine, apigenin, rhamnetin, apigetrin and a new flavone, apigenin 7-O-β-D(-6″-*p*-coumaroyl)-glucoside, were isolated from the aerial part of patchouli, *Pogostemon cablin* (Labiatae). These compounds were identified by analysis of various spectral data.

Keywords——*Pogostemon cablin*; patchouli; Labiatae; flavonoids; pachypodol; ombuine; rhamnetin; apigenin; apigetrin; apigenin 7-O-β-D(-6″-*p*-coumaroyl)-glucoside

Patchouli, *Pogostemon cablin* BENTHAM (Labiatae), is cultivated in tropical Asia in order to obtain the essential oil (patchouli oil). Previous studies on the constituents of patchouli oil have shown the presence of mono- and sesquiterpenoids[2-4] and alkaloids.[5] In this paper, we report on the flavonoids isolated from the aerial part of patchouli.

Pachypodol (I)[6] and ombuine (II)[7,8] were isolated from the *n*-hexane extract, and ombuine (II), apigenin (III),[9,10] rhamnetin (IV),[10] a new flavone (V) and apigetrin (VI)[10,11] from the ethyl acetate extract, by silica gel column chromatography. The flavonoids I—IV and VI were identified by comparing various spectral data with those in the literature. The compound V gave positive HCl/Mg and HCl/Zn tests, and its characteristic fragments in the mass spectrum (MS) suggested it to be an apigenin derivative.[12] The ultraviolet (UV) spectrum of (V) in methanol exhibited maxima at 270 nm and 318 nm. The bathochromic shifts of the UV absorption band I with NaOH and $AlCl_3$ also suggested the presence of 4′- and 5-hydroxyl groups, and the absence of an NaOAc band II shift indicated that the 7-hydroxyl group was substituted. The infrared (IR) spectrum indicated the presence of an α,β-unsaturated carbonyl group at 1690 cm^{-1} other than the 4-positional carbonyl group of flavone. The nuclear magnetic resonance (NMR) spectrum exhibited doublet signals of $J=$ 16 Hz at δ 6.32 and 7.50, $J=9$ Hz at δ 6.68, 7.34, 6.93 and 7.94, and $J=2$ Hz at δ 6.48 and 6.84, suggesting the presence of two 1,4- and one 1,2,3,5-substituted benzenes, and one *trans* disubstituted ethylene conjugated with the carbonyl group. Upon the hydrolysis of (V), apigenin, *p*-coumaric acid and glucose were identified by thin-layer chromatography (TLC). On the basis of the above results, it was assumed that the compound V contained *p*-coumaroyl glucose linked at the 7-hydroxyl group of apigenin. Further, we attempted to use ^{13}C-NMR in order

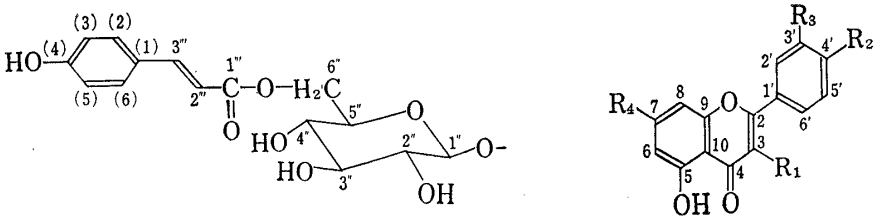

Fig. 1

I : R_2=OH, R_1,R_3,R_4=OMe
II : R_1,R_3=OH, $R_2$$R_4$=OMe
III : R_1,R_3=H, R_2,R_4=OH
IV : R_1,R_2,R_3=OH, R_4=OMe
V : R_1,R_3=H, R_2=OH,R_4=O-(6″-*p*-coumaroyl)-glu
VI : R_1,R_3=H, R_2=OH, R_4=O-glu
VII : R_1,R_3=H, R_2=OH, R_4=O-(6″-acetyl)-glu
VIII : R_1,R_3=H, R_2=O-*p*-coumaroyl, R_4: O-glu

Table I. ^{13}C Chemical Shifts of Flavonoids isolated from Patchouli

	I	II	III	IV	V	VI	VII
2	155.8[a]	147.4	164.1	147.3	162.7	162.9	162.81
3	137.9	136.2	102.9	136.0	103.0	103.1	103.24
4	178.0	176.0	181.7	175.9	181.9	181.7	182.03
5	156.3[a]	156.1	157.3	156.0	156.8	156.9	157.00
6	97.8	97.5	98.9	97.4	99.5	99.6	99.77
7	165.1	164.9	163.7	164.8	164.3	164.3	164.39
8	92.4	92.0	94.0	91.8	94.7	94.9	94.94
9	160.9	160.3	161.5[b]	160.3	161.3[c]	161.4[d]	161.45[e]
10	105.2	104.1	103.8	104.0	105.3	105.4	105.55
1'	120.7	122.0	121.3	121.9	121.0	121.0	121.17
2'	112.1	115.6	128.5	115.3	128.5	128.6	128.59
3'	147.5	147.0	116.0	145.1	116.0	116.1	116.08
4'	150.0	149.0	161.1[b]	147.8	161.1[c]	161.1[d]	161.22[e]
5'	115.7	111.8	116.0	115.6	116.0	116.1	116.08
6'	122.3	122.0	128.5	120.0	128.5	128.6	128.59
1''					99.5	100.0	99.77
2''					73.0	73.2	73.13
3''					76.3	77.2	76.32
4''					70.0	70.0	69.95
5''					73.8	76.5	74.02
6''					63.4	60.7	63.46
3-OMe	59.7					Acetyl (CH_3)	20.53
7-OMe	56.2[f]	56.0[g]		56.0		Acetyl (CO)	170.19
3'-OMe	55.9[f]						
4'-OMe		55.9[g]				*p*-Coumaric acid	
1'''					166.4	170.7	
2'''					113.7	116.0	
3'''					144.9	146.7	
(1)					124.9	126.9	
(2), (6)					131.7	131.7	
(3), (5)					115.7	117.3	
(4)					159.7	160.3	

The measurements were made on a JEOL FX-100 spectrometer in DMSO-d_6 with TMS as an internal reference and are expressed in terms of ppm. The chemical shifts of VII are cited from reference 14 and those of *p*-coumaric acid from reference 9.
a—*g*) The assignments may be reversed.

to determine the position of the *p*-coumaroyl group in the glucose moiety.[13] The ^{13}C-NMR spectral data for (I)—(VI), apigenin 7-O-β-D-(-6''-acetyl)-glucoside (VII)[14] and *p*-coumaric acid[9] are given in Table I. As can be seen from Table I, the 6''-positional carbon signal of glucose of apigetrin (VI) at δ 60.7 was shifted downfield to δ 63.4 in compound V. Its chemical shift was exactly the same as that of (VII).[14] Consequently, the new flavone V was identified as apigenin 7-O-β-D(-6''-*p*-coumaroyl)-glucoside.

The physical data for (V) were similar to those for quinqueloside (VIII), which was previously isolated from *Leonuris quinquelobatus*.[15] It is interesting in connection with the chemotaxonomy of Labiatae that quinqueloside possesses a *p*-coumaroyl group at the 4'-hydroxyl group of the B-ring of apigetrin, whereas the new flavone V isolated from patchouli has this group at the 6''-position of glucose.

Experimental

Melting points were recorded on a Yanagimoto micro melting point apparatus and are uncorrected. Spectra were obtained on the following machines: UV on a Shimadzu UV-210, PMR on a JEOL JNM-PS-100, CMR on a JEOL FX-100, MS on a Hitachi RMU-7L and IR on a Hitachi 260-10. TLC was carried out on 0.25 mm silica gel plates ($60F_{254}$, Merck) with $CHCl_3$–MeOH–H_2O (5:6:4). *Rf* values: (I) 0.77, (II) 0.69, (III) 0.53, (IV) 0.38, (V) 0.15, (VI) 0.10.

Extraction and Isolation——The aerial part, cut into pieces, of *Pogostemon cablin* was purchased from Kinokuniya Kan-Yakkyoku Co., Ltd., Japan. The crude drug (10 kg) was extracted with n-C_6H_{14} (18 l) and next with MeOH (18 l). The extractions were repeated twice. The MeOH extract was concentrated to 500 ml and dissolved in 5 l of H_2O. The solution was extracted with 18 l of EtOAc in a separatory funnel. In all, 310 g of n-C_6H_{14} and 200 g of EtOAc extracts were obtained. The extracts were each subjected to column chromatography on silica gel with n-C_6H_{14}–EtOAc–MeOH as an eluent. The physical data, except for ^{13}C-NMR, of isolated compounds were as follows; pachypodol (I), yellowish needles (148 mg), mp 164—167°, UV λ_{max}^{MeOH} nm: 256, 358, $\lambda_{max}^{MeOH+NaOH}$ nm: 265, 417, $\lambda_{max}^{MeOH+AlCl_3}$ nm: 268, 298, 373, 409, $\lambda_{max}^{MeOH+NaOAc}$ nm: 256, 408, MS m/z (%): 344 (M^+, 100), 343 (49), 329 (39), 313 (12), 301 (40), 167 (15), 151 (12), NMR (DMSO-d_6, δ, ppm): 3.84, 3.87 and 3.89 (3H, s, 3′-, 7- and 3-OMe, respectively), 6.34 (1H, d, J=2 Hz, 6-H), 6.74 (1H, d, J=2 Hz, 8-H), 6.95 (1H, d, J=8 Hz, 5′-H), 7.61 (1H, dd, J=2, 8 Hz, 6′-H), 7.66 (1H, d, J=2 Hz, 2′-H), ombuine (II), pale yellowish needles (1.3 g), mp 221—223°, UV λ_{max}^{MeOH} nm: 255, 268 (sh), 371, $\lambda_{max}^{MeOH+NaOH}$ nm: 264, 419, $\lambda_{max}^{MeOH+AlCl_3}$ nm: 264, 300 (sh), 365, 427, $\lambda_{max}^{MeOH+NaOAc}$ nm: 255, 272 (sh), 373, 423 (sh), MS m/z (%): 330 (M^+, 100), 329 (14), 287 (9), 167 (6), 151 (10), NMR (DMSO-d_6, δ, ppm): 3.88 (6H, s, 4′- and 7-OMe), 6.30 (1H, d, J=2 Hz, 6-H), 6.70 (1H, d, J=2 Hz, 8-H), 6.92 (1H, d, J=9 Hz, 5′-H), 7.72 (1H, dd, J=2, 9 Hz, 6′-H), 7.76 (1H, d, J=2 Hz, 2′-H), apigenin (III), a pale yellowish powder (313 mg), mp >300°, UV λ_{max}^{MeOH} nm: 268, 305 (sh), 334, $\lambda_{max}^{MeOH+NaOH}$ nm: 275, 299, 359, 383, $\lambda_{max}^{MeOH+AlCl_3}$ nm: 275, 303, 349, 384, $\lambda_{max}^{MeOH+NaOAc}$ nm: 275, 300, 358, MS m/z (%): 270 (M^+, 100), 269 (9), 242 (9), 153 (26), 152 (16), 121 (17), 118 (11), NMR (DMSO-d_6, δ, ppm): 6.22 (1H, d, J=2 Hz, 6-H), 6.50 (1H, d, J=2 Hz, 8-H), 6.76 (1H, s, 3-H), 6.96 (2H, d, J=9 Hz, 3′- and 5′-H), 7.93 (2H, d, J=9 Hz, 2′- and 6′-H), rhamnetin (IV): yellowish needles (116 mg), mp 280—285°, UV λ_{max}^{MeOH} nm: 257, 369, $\lambda_{max}^{MeOH+NaOH}$ nm: 263, 416, $\lambda_{max}^{MeOH+AlCl_3}$ nm: 271, 343 (sh), 445, $\lambda_{max}^{MeOH+NaOAc}$ nm: 258, 271 (sh), 378 (sh), 420, MS m/z (%): 316 (M^+, 100), 315 (12), 273 (11), 167 (9), 137 (28), NMR (DMSO-d_6, δ, ppm): 3.88 (3H, s, 7-OMe), 6.34 (1H, d, J=2 Hz, 6-H), 6.90 (1H, d, J=2 Hz, 8-H), 6.90 (1H, d, J=8 Hz, 5′-H), 7.59 (1H, dd, J=2, 8 Hz, 6′-H), 7.73 (1H, d, J=2 Hz, 2′-H), apigenin-7-O-β-D(-6″-p-coumaroyl)-glucoside (V), a pale yellowish powder (196 mg), mp 260—264°, $[\alpha]_D^{25}$ −135° (c=0.21), UV λ_{max}^{MeOH} nm: 270, 304 (sh), 318, $\lambda_{max}^{MeOH+NaOH}$ nm: 268, 298 (sh), 317, 382, $\lambda_{max}^{MeOH+AlCl_3}$ nm: 279, 301, 323, 383, $\lambda_{max}^{MeOH+NaOH}$ nm: 270, 301 (sh), 318, 379, IR ν_{max}^{KBr} cm^{-1}: 3300—3500 (OH), 1655 and 1690 (C–O), MS m/z (%): 270 (100), 242 (10), 153 (27), 152 (18), 121 (23), 118 (14), NMR (DMSO-d_6, δ, ppm): 5.11 (1H, d, J=6 Hz, 1″-H), 6.32 (1H, d, J=16 Hz, 2‴-H), 6.48 (1H, d, J=2 Hz, 6-H), 6.68 (2H, d, J=9 Hz, (3)- and (5)-H), 6.84 (1H, d, J=2 Hz, 8-H), 6.93 (2H, d, J=9 Hz, 3′- and 5′-H), 6.80 (1H, s, 3-H), 7.34 (2H, d, J=9 Hz, (2)- and (6)-H), 7.50 (1H, d, J=16 Hz, 3‴-H), 7.94 (2H, d, J=9 Hz, 2′- and 6′-H), 12.88 (1H, s, 5-OH), apigetrin (VI), pale yellowish needles (94 mg), mp 218—220°, $[\alpha]_D^{25}$ −80° (c=0.13), UV λ_{max}^{MeOH} nm: 268, 333, $\lambda_{max}^{MeOH+NaOH}$ nm: 250, 267, 295 (sh), 388, $\lambda_{max}^{MeOH+AlCl_3}$ nm: 277, 300, 345, 382, $\lambda_{max}^{MeOH+NaOAc}$ nm: 257 (sh), 268, 341, 391, MS m/z (%): 270 (100), 242 (11), 153 (25), 152 (16), 121 (22), 118 (15), NMR (DMSO-d_6, δ, ppm): 6.50 (1H, d, J=2 Hz, 6-H), 6.88 (1H, d, J=2 Hz, 8-H), 6.90 (1H, s, 3-H), 7.01 (2H, d, J=9 Hz, 3′- and 5′-H), 8.03 (2H, d, J=9 Hz, 2′- and 6′-H).

Hydrolysis of V——Compound V (10 mg) was dissolved in 5% aqueous H_2SO_4 (10 ml) and refluxed for 3 hours. From the water-insoluble fraction, p-coumaric acid (Rf 0.13) and apigenin (Rf 0.38) were identified by TLC (0.25 mm silica gel plate 60F_{254} (Merck), lower layer of $CHCl_3$–MeOH–H_2O (65: 35: 40)). The mother liquor was neutralized with a saturated aquoeus solution of $Ba(OH)_2$ and the dextrorotatory filtrate was evaporated to a small volume. Glucose (Rf 0.34) was detected as the sugar component of V by TLC (0.25 mm silica gel plate 60F_{254} (Merck), n-BuOH–Me_2CO–H_2O (4: 5: 1)).

References and Notes

1) Location: *1432-1 Horinouchi, Hachioji, Tokyo, 192-03 Japan.*
2) S.J. Terhune, J.W. Hogg, and B.M. Laurence, *Tetrahedron Lett.*, **1973**, 4705.
3) H. Hikino and K. Ito, *Chem. Pharm. Bull.*, **16**, 1608 (1968).
4) N. Tsubaki, K. Nishimura, and Y. Hirose, *Bull. Chem. Soc. Jpn.*, **40**, 597 (1967).
5) G. Buchi, I.M. Goldman, and D.W. Mayo, *J. Am. Chem. Soc.*, **88**, 3109 (1966).
6) A.G. Valesi, *Phytochemistry*, **11**, 2821 (1972).
7) L. Jurd, *J. Org. Chem.*, **27**, 1294 (1962).
8) H. Wagner and V.M. Chari, *Tetrahedron Lett.*, **1976**, 1799.
9) B. Ternai and K.R. Markham, *Tetrahedron*, **32**, 565 (1976).
10) T.J. Mabry, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer, New York, 1970.
11) V.M. Chari, M. Jordan, H. Wagner, and P.W. Thies, Phytochemistry, **16**, 1110 (1977).
12) J.B. Harborne, T.J. Mabry, and H. Mabry, "The Flavonoids," Chapman and Hall, London, 1975.
13) K. Yoshimoto, Y. Itatani, and Y. Tsuda, *Chem. Pharm. Bull.*, **28**, 2065 (1980).
14) R. Kunde and O. Isaac, *Planta Medica*, **37**, 124 (1979).
15) V.V. Petrenko, *Chem. Abstr.*, **64**, 15827*c* (1966).