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Constituents from *Syzygium aromaticum* Merr. *et* Perry

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Abstract – From the dried flower-buds of *Syzygium aromaticum* Merr. et Perry (Myrtaceae), seven compounds, *i.e.*, eugenol (**1**), oleanolic acid (**2**), kaempferol 7-*O*-metylether (**3**), 3,3',4-tri-*O*-methylellagic acid (**4**), maslinic acid (**5**), β-sitosterol-3-*O*-glucoside (**6**), and isorhamnetin 3-*O*-glucoside (**7**) were isolated. Compound **1** showed cyclooxygenase-2 (COX-2) inhibitory activity.

Key words – *Syzygium aromaticum*, Myrtaceae, eugenol, kaempferol 7-*O*-methylether, 3,3',4-tri-*O*-methylellagic acid, isorhamnetin 3-*O*-glucoside, COX-2 inhibitory activity.

Introduction

Clove (the dried flower-buds of *Syzygium aromaticum*) is an oriental drug which has been used as vermifuge, antibacterial agent and to treat toothache (Shanghai Science and Technologic Publisher and Shougakukan, 1988). Various compounds, such as tannins and triterpenoids were reported from this plant (Tanaka *et al.*, 1993, Umehara *et al.*, 1992). In the course of our effort to isolate antiinflammatory compounds from natural sources, the EtOAc soluble fraction of clove significantly inhibited cyclooxygenase-2 (COX-2) and chromatographic isolation of this fraction was conducted.

This paper describes the isolation, structure elucidation of seven compounds from the EtOAc fraction and the COX-2 inhibitory activity of **1**.

Experimental

General experimental procedures – The mps were taken on a Yanaco micro-melting point apparatus and are uncorrected. The IR spectra were determined in KBr tablets on a Mattson Polaris TM (FT-IR) spectrometer and the UV spectra were run with a Varian DMS 200 UV-Vis spectrophotometer. The EI-MS and FAB-MS spectra were recorded on a JMS SX-102A and JMS HX-110/110A (JEOL) spectrometer. The ^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker DRX-500 or Bruker AMX-300 spectrometer with TMS as an intenal standard and chemical shifts are given a ppm. TLC chromatography was performed on precoated Kieselgel 60 F_{254} plates (Merck, 5715)

Plant material – The dried flower-buds of *S. aromaticum* were purchased from a crude drug market in Andong.

Extraction and isolation – The chopped cloves (1.2 kg) were extracted with MeOH under reflux (65°C, three times, 6h each). The combined MeOH extracts were evaporated under reduced pressure, to give a residue, which was partitioned with *n*-hexane, EtOAc, *n*-BuOH and water, successive-

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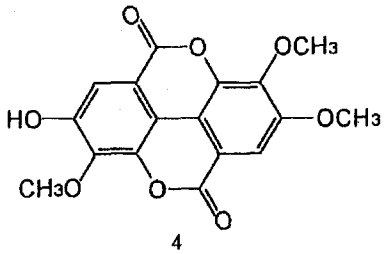

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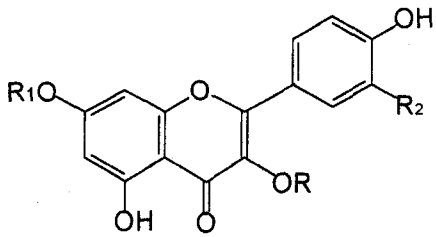

3 R=R_2=H, R_1=CH_3
7 R=Glucose, R_1=H, R_2=OCH_3

ly. EtOAc fraction (50 g) was chromatographed on silica gel with *n*-hexane-EtOAc (gradient), $CHCl_3$-MeOH (10:1), $CHCl_3$-MeOH (8:1), $CHCl_3$-MeOH-H_2O (8:2:0.5), $CHCl_3$-MeOH-H_2O (7:3:1), $CHCl_3$-MeOH-H_2O (52:28:8), as eluents to give sixteen subfractions. The subfraction **1** was chromatographed on silica gel eluting with $CHCl_3$-MeOH (10:0.5), to give **1**. The subfraction **5** was chromatographed on silica gel column with $CHCl_3$-MeOH (99.5:0.5), to give **2** and **3**. With same some manner as above, **4** from subfraction **8** ($CHCl_3$-MeOH=99:1), **5** from subfraction **9** and **10** ($CHCl_3$-MeOH-H_2O=8:2:0.5), **6** from subfraction **11** ($CHCl_3$-MeOH-H_2O=8:2:0.5) and **7** from subfraction **12** ($CHCl_3$-MeOH-H_2O=8:2:0.5), followed by RP-18 chromatography (MeOH-H_2O=1:1) were isolated respectively.

Compound 1 – colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 6.85 (1H, d, J=8.7 Hz, H-6), 6.70-6.67 (2H, m, H-3 and 5), 6.02-5.89 (1H, m, β-H), 5.07 (1H, dd, J=18.1 and 1.7 Hz, γ-H_A), 5.06 (1H, dd, J=11.2 and 1.7 Hz, γ-H_B), 3.87 (3H, s, OCH_3), 3.32 (2H, d, J=6.7 Hz, α-H_2). ^{13}C-NMR ($CDCl_3$) δ: 146.8 (C-1), 144.3 (C-2), 138.2 (β-C) 132.3 (C-4), 121.6 (C-6), 115.9 (γ-C), 114.7 (C-3), 111.5 (C-5), 56.3 (OCH_3), 40.3 (α-C).

Compound 2 – white powder from MeOH, mp 300-303°C, LB test: positive. IR, ν_{Max} 3440 (OH), 1701 (C=O), 1636 (C=C), 1028 cm^{-1}. EI-MS m/z (rel. int.) 456 $[M]^+$ (10), 410 $[M-COOH+H]^+$ (2), 248 [D/E ring]$^+$ (51), 203 [D/E ring-COOH]$^+$ (26), 175 (4), 133 (15), 57 (100). ^{1}H-NMR (pyridine-d_5) δ: 5.48 (1H, brs, H-12), 3.43 (1H, dd, J=5.7 and 9.7 Hz, H-3), 3.28 (1H, dd, J=4.0 and 13.4 Hz, H-18), 1.26, 1.22, 1.01, 1.01, 0.99, 0.93, 0.88 (3H each, s, CH_3). ^{13}C-NMR (pyridine-d_5): see Table 1.

Compound 3 – Yellow needles from MeOH, mp 225-227°C, $FeCl_3$, Mg/HCl test:

Table 1. ^{13}C-NMR chemical shifts of Compounds **2**, **5**, and **6** in pyridine-d_5

Carbon No.	Compound		
	2	**5**	**6**
C-1	38.9	46.7	37.5
C-2	28.3	68.6	30.3
C-3	78.1	83.8	78.1
C-4	39.3	39.8	39.9
C-5	55.8	55.9	140.9
C-6	18.8	18.9	121.9
C-7	33.2	33.3	32.2
C-8	39.7	39.8	32.1
C-9	48.1	48.2	50.4
C-10	37.3	38.6	37.0
C-11	23.7	23.8	21.3
C-12	122.5	122.5	39.4
C-13	144.8	144.9	42.5
C-14	42.1	42.2	56.9
C-15	28.0	28.3	24.5
C-16	23.8	23.9	28.6
C-17	46.7	47.8	56.3
C-18	42.0	42.0	12.0
C-19	46.5	46.4	19.4
C-20	30.9	31.0	36.4
C-21	34.2	34.2	19.0
C-22	33.2	33.3	34.2
C-23	28.7	29.3	26.4
C-24	16.5	17.5	46.1
C-25	15.5	16.9	29.5
C-26	17.4	17.7	19.2
C-27	26.1	26.2	20.0
C-28	180.2	180.2	23.4
C-29	33.2	33.3	12.2
C-30	23.7	23.7	
C-1′			102.6
C-2′			75.4
C-3′			78.6
C-4′			71.7
C-5′			78.5
C-6′			62.9

positive. IR ν_{max} 3260 (OH), 1663 (α,β-unsaturated C=O), 1609 1587, 1504 (C=C), 1233, 1165, 817 cm^{-1}; UV λ_{max} (MeOH) 268, 293 (sh), 326 (sh), 365 nm; (MeONa) 246, 271, 335 (sh), 416 nm; ($AlCl_3$) 260 (sh), 272, 305 (sh), 356, 424 nm; ($AlCl_3$+HCl) 261 (sh), 304 (sh), 352, 423 nm; (NaOAc) 267, 328 (sh), 384 nm; (NaOAc+H_3BO_3) 268, 296 (sh), 325 (sh), 368 nm. EI-MS m/z (rel. int.) 300 $[M]^+$ (100), 299 $[M-H]^+$ (31), 285 $[M-CH_3]^+$ (2), 271 $[M-HCO]^+$ (13), 257 $[M-CH_3-CO]^+$ (16), 229 $[M-CH_3-2CO]^+$ (4), 167 $[A_1+H]^+$ (3), 121 $[B_2]^+$ (28), 105 (5). ^{1}H-NMR (DMSO-d_6) δ: 8.08 (2H, d, J=8.8 Hz, H-2', 6'), 6.93 (2H, d, J=8.8 Hz, H-3', 5'), 6.72 (1H, d, J=2.0 Hz, H-8), 6.34 (1H, d, J=2.0 Hz, H-6), 3.85 (3H, s, OCH_3). ^{13}C-NMR (DMSO-d_6) δ 147.2 (C-2), 136.0 (C-3), 176.0 (C-4), 160.3 (C-5), 97.4 (C-6), 164.9 (C-7), 92.0 (C-8), 156.1 (C-9), 104.0 (C-10), 121.6 (C-1'), 129.6 (C-2'), 115.7 (C-3'), 159.3 (C-4'), 115.7 (C-5'), 129.6 (C-6').

Compound 4 – pale yellow needles from MeOH, mp 284-286°C. UV λ_{max} (EtOH) 249, 290 (sh), 360 (sh), 373 nm; (EtOH+NaOH) 237 (sh), 268 (sh), 315, 414 nm. IR ν_{max} (KBr) 3440, 1752 (lactone), 1609, 1360 cm^{-1}. EI-MS m/z (rel. int.) 344 $[M]^+$ (100), 329 $[M-CH_3]^+$ (17), 301 $[M-CH_3-CO]^+$ (8), 286 $[M-2CH_3-CO]^+$ (18), 268 $[M-2CH_3-2CO]^+$ (6). ^{1}H-NMR (DMSO-d_6) δ: 7.63 (1H, s, H-5'), 7.54 (1H, s, H-5), 4.05, 4.04, 4.00 (3H each, s, 3, 3', 4-OCH_3). ^{13}C-NMR (DMSO-d_6) δ: 111.9 (C-1), 140.8 (C-2), 141.5 (C-3), 153.8 (C-4), 107.5 (C-5), 113.4 (C-6), 158.5 (C-7), 111.2 (C-1'), 141.0 (C-2'), 140.2 (C-3'), 152.6 (C-4'), 111.6 (C-5'), 112.5 (C-6'), 158.3 (C-7') 61.3, 61.0, 56.7 (OCH_3 each).

Compound 5 – white needles from MeOH, mp 276-278°C, LB test: positive. IR ν_{max} 3418 (OH), 1698 (C=O), 1634 (C=C), 1049 cm^{-1}. EI-MS m/z (rel. int.) 427 $[M]^+$ (3), 428 $[M-COO]^+$ (2), 248 [D/E ring]$^+$ (28), 203 [D/E ring-COOH]$^+$ (10), 189 (2) 55 (100). ^{1}H-NMR (pyridine-d_5) δ: 5.46 (1H, brs, H-12), 4.07 (1H, ddd, J=4.5, 9.0 and 11.2 Hz, H-2), 3.38 (1H, d, J=9.3 Hz, H-3), 3.29 (1H, dd, J=4.0 and 13.0 Hz, H-18). ^{13}C-NMR (pyridine-d_5): see Table 1.

Compound 6 – white amorphous powder from MeOH, mp 298-299°C, LB test, Molisch test: positive. ^{1}H-NMR (pyridine-d_5) δ: 5.35 (1H, brd, J=4.5 Hz, H-6), 5.04 (1H, d, J=7.7 Hz, anomeric H), 0.98 (3H, d, J=6.4 Hz, 21-CH_3), 0.93 (3H, s, 19-CH_3), 0.89, 0.87, 0.84 (3H each, s, 26, 27, 29-CH_3), 0.66 (3H, s, 18-CH_3). ^{13}C-NMR (pyridine-d_5): see Table 1.

Compound 7 – yellow needles from MeOH, mp 170-175°C $FeCl_3$, Mg/HCl, Molisch: positive. IR ν_{max} 3409 (OH), 1653 (α,β-unsaturated C=O), 1607, 1501 (C=C), 1294, 1057 cm^{-1}; UV λ_{max} (MeOH) 258, 266, 303 (sh), 353 nm; (MeONa) 275, 330, 407 nm; ($AlCl_3$) 273, 305, 361 (sh), 400 nm; ($AlCl_3$+HCl) 273, 302, 359, 401 nm; (NaOAc) 276, 317, 375 nm; (NaOAc+H_3BO_3) 259, 268 (sh), 302 (sh), 357 nm; FAB-MS m/z 479 $[M+H]^+$. ^{1}H-NMR (DMSO-d_6) δ: 7.94 (1H, d, J=2.0 Hz, H-2'), 7.50 (1H, dd, J=8.4 and 2.0 Hz, H-6'), 6.92 (1H, d, J=8.4 Hz, H-5'), 6.43 (1H, d, J=2.1 Hz, H-8), 6.21 (1H, d, J=2.1 Hz, H-6), 5.56 (1H, d, J=7.4 Hz, anomeric H), 3.77 (3H, s, OCH_3). ^{13}C-NMR (DMSO-d_6) δ 156.4 (C-2), 133.0 (C-3), 177.5 (C-4), 161.2 (C-5), 98.7 (C-6), 164.1 (C-7), 93.7 (C-8), 156.3 (C-9), 104.0 (C-10), 121.1 (C-1'), 115.2 (C-2'), 149.4 (C-3'), 146.9 (C-4'), 113.5 (C-5'), 122.0 (C-6'), 100.9 (C-1"), 74.3 (C-2"), 77.4 (C-3"), 69.8 (C-4") 76.4 (C-5"), 60.6 (C-6"), 55.7 (OCH_3).

COX-2 inhibition test – Inhibition of COX-2 activity by sample was evaluated by inhibition of PGD_2 production in the BMMC. BMMC were washed once and suspended at a cell density of 0.5×10^6 cells/ml in enriched medium, and activated with KL (100 ng/ml), IL-10 (100 U/ml) and LPS (100 ng/ml) in the presence or absence of sample in DMSO with the final concentration of 1% using a DMSO-cell control at 37°C for 8 h by preincubation the cells with 10 μg/ml aspirin for 2 h to irreversibly inactivate preexisting COX-1. Reactions were stopped by centrifugation at $120\times g$ for 5 min at 4°C. The supernat-

ants were retained for assay of PGD_2. PGD_2 generation by BMMC was assayed using a PGD_2 assay kit (Amersham, Buckinghamshire, UK).

Results and Discussion

Repeated silica gel and RP-18 column chromatography of EtOAc soluble fraction afforded seven compounds, four of which were identified as eugenol **1**, oleanolic acid **2**, maslinic acid **5** and β-sitosterol-3-*O*-glucoside **6** by comparison of spectral data with those of the reported data and direct comparison with authentic standards (Brieskorn *et al.*, 1975).

Compound **3** gave positive color reaction for flavonoids and exhibited UV spectrum similar to those of flavonols. Its IR spectrum showed absorption bands due to an α, β-unsaturated C=O (1663 cm^{-1}) and hydroxyl groups (3260 cm^{-1}). The MS spectrum revealed the molecular ion peak at m/z 300 and the characteristic fragment ion peaks at m/z 167 and 121 by the *retro*-Diels Alder fragmentation of flavonoid. The ^{1}H-NMR spectrum of **3** exhibited one methoxyl signal at δ 3.85 and two *meta* coupled signals due to H-6 and H-8 at δ 6.34 (1H, d, *J*= 2.0 Hz) and 6.72 (1H, d, *J*=2.0 Hz). And two signals at δ 8.08 (2H, d, *J*=8.8 Hz) and 6.93 (2H, d, *J*=8.8 Hz) were resolved into AA'BB' system due to a *para*-substituted benzene ring. Thus, **3** is a kaempferol derivative having one methyl group on C_5-OH or C_7-OH. Since the sbsorption band II of **3** was observed at 268 nm in its UV spectrum, one methyl group should be located on C_7-OH. From the above results, **3** was identified as kaempferol 7-*O*-methylether and the ^{13}C-NMR data supported this conclusion (Agrawal, 1989).

Compound **4** gave a positive color reaction to $FeCl_3$ and showed a molecular ion peat at m/z 344 in the MS spectrum. Its IR spectrum suggested the absorption band due to lactone moiety. The ^{1}H-NMR spectrum showed three methoxyl groups (δ 4.05, 4.04, 4.00) and two aromatic protons (δ 7.63 and 7.54). On the basis of above data, **4** was supposed to be tri-*O*-methylellagic acid. The structure of **4** was identified as 3,3',4-tri-*O*-methylellagic acid by comparison of data in literature (Terashima *et al.*, 1990).

Compound **7** gave positive color reactions for flavonoid glycoside and showed typical UV spectrum due to 5,7,4'-trihydroxyflavonol glycoside. Its molecular weight was deduced to be 478 by the pseudomolecular ion peak at m/z 479 $[M+H]^+$ in the FAB-MS spectrum. ^{1}H-NMR spectrum of **7** revealed two *mata*-coupled signals at δ 6.21 (1H, d, *J*=2.1 Hz, H-6) and 6.43 (1H, d, *J*=2.1 Hz, H-8), three ABX type signals due to B ring protons at δ 7.94 (1H, d, *J*=2.0 Hz, H-2′), 7.50 (1H, dd, *J*=8.4 and 2.0 Hz, H-6′) and 6.92 (1H, d, *J*=8.4 Hz, H-5′). Furthermore, one anomeric doublet (δ 5.56, *J*=7.4 Hz) and one methoxyl singlet were also observed. In the light of above findings, **7** was supposed to be an isorhamnetin monoglycoside. In the ^{13}C-NMR spectrum, one set of signals owing to glucosyl moiety were observed. On the comparison of the ^{13}C-NMR chemical shift of **7** with those of isorhamnetin, the signals corresponding to C-2 and C-3 of **7** revealed glycosidation shifts at δ 156.4 (+9.3 ppm) and 133.0 (−6.1 ppm), suggesting that glucose unit was attached at C-3 of isorhamnetin (Harborne and Mabry, 1982). Thus, the structure of **7** was identified as isorhamnetin 3-*O*-β-D-glucopyranoside.

When each isolated compounds were subject to COX-2 inhibitory system, only **1** inhibited the activity of this enzyme (95%, final concentration: 2.5 μg/ml).

Acknowledgements

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