Arch. Pharm. Res. 10(1), 9-13 (1987)

Soyasapogenols B and E from *Melilotus officinalis*

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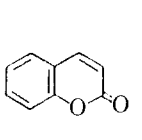
(Received January 7, 1987)

Abstract □ From the aerial parts of *Melilotus officinalis* (Leguminosae) soyasapogenols B, mp 259-260°, and E, mp 246-247°, were isolated along with coumarin and kaempferol. This is the first report of the isolation of soyasapogenols from the genus Melilotus.

Keywords □ *Melilotus officinalis*, Leguminosae, Soyasapogenol B, Soyasapogenol E.

Melilotus extract obtained from *Melilotus officinalis* Lam.(Leguminosae) has been used as a herbal remedy for arthritis, brachialgia, bronchitis, hemorrhoids, rheumatism and phlebitis in Europe[1] and China[2] and still used for the treatment of hemorrhoids, phlebitis and inflammation in Korea. It has been reported that the plant contains coumarins, flavonoids, and amines and the active component is coumaric acid derivative, melilotoside[2-4]. Further investigation on this plant showed some flavonoid glycosides and saponins, of which the latter were also responsible for the anti-inflammatory activity[5]. This paper deals with the chemical elucidation of triterpenoid sapogenols.

On acid hydrolysis, the BuOH fraction that was obtained from a methanolic extract of aerial parts of *M. officinalis* afforded a mixture of aglycones. The mixture was chromatographed repeatedly on a silica gel flash column and purified by recrystallization to give compounds **1**, **2**, **3** and **4**. Among these compounds, compounds **1**, mp 67-8°, and **2**, mp 276-8°, were identified as coumarin and kaempferol by direct comparison with authentic samples. Compounds **3** and **4** showed positive results in Liebermann-Burchard and tetranitromethane tests. Compound **3**, mp 259-260°, showed IR absorption bands at 3400 cm^{-1} and 1650, 810, and 800 cm^{-1} due to hydroxyl and double bond, respectively. It gave a triacetate (**5**), mp 180-1°, upon acetylation. Compounds **3** and **5** exhibited prominent mass spectral peaks at *m/z* 234 and 276, respectively, due to a retro Diels-Alder cleavage of ring C in each compound[6]. The characteristic fragments corresponding to the release of H_2O and H_2O+CH_2OH



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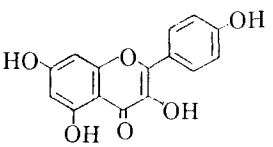

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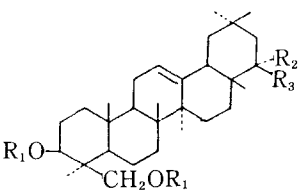

3 $R_1=R_2=H$; $R_3=OH$
4 $R_1=H$; R_2, $R_3=O$
5 $R_1=Ac$; $R_2=H$; $R_3=OAc$

moieties from rings A/B and of H_2O from rings D/E of **3** were also showed at *m/z* 206, 175 and 216, respectively. This evidence suggested that one primary hydroxyl and one of two secondary hydroxyl groups were located at either ring A or B and the second hydroxyl group at rings D or E[6]. This was further supported by the prominent mass peaks in **5** at *m/z* 247, 174 and 216. Seven tertiary methyl singlets (δ 0.86-1.24) and an olefinic proton at δ 5.24 in the NMR spectrum of **3** as well as the above mass spectral data supported the olean-12-en assignment. The 3β-and 24-hydroxyl functionalities were suggested by NMR: δ 3.35 (1H, m), 4.20 (1H, d, J=11.1Hz) and 3.33 (1H, d, J=11.1Hz)[7,8]. The assignment of the remaining secondary hydroxyl group in rings D/E was allocated to C-21α or C-22β position on

the basis of its chemical shift and coupling constants [δ 3.35(m) in **3** and 4.64(t, J=3.6Hz) in **5**] and easy acetylation of the hydroxyl group under mild condition. The properties of **3** and **5** closely resemble those reported for 3β, 22β, 24-trihydroxyolean-12-ene (=soyasapogenol B) and its acetate[9], respectively, and therefore **3** is most likely identical with soyasapogenol B. Furthermore, the found values of the chemical shifts of the angular methyl groups in **5** were in excellant agreement with those calculated for 3β, 22β, 24-trihydroxyolean-12-ene as shown in Table I. Therefore the structure of **3** was assigned as soyasapogenol B. In addition, direct comparison of **3** with an authentic sample of soyasapogenol B clearly supported this assignment.

Compound **4**, mp 246-7°, showed IR absorption band at 1706 cm^{-1} due to carbonyl group along with hydroxyl and double bond as indicated in **3**. The mass spectrum of **4** showed a molecular ion peak at *m/z* 456 in addition to typical retro Diels-Alder fragmentation peaks at *m/z* 232 (D/E rings) and 224 (A/B rings). The ion peaks at *m/z* 456 and 232 were 2 mass units smaller than analogous fragments for **3** at *m/z* 458 and 234, respectively. This information suggested that the carbonyl group of **4** was located at rings D/E and the structure of rings A/B residue was identical with that of **3**. The presence of a set of doublets at δ 4.21 and 3.33 with J=11Hz and a triplet at δ 3.42 (J=8Hz) in the NMR spectrum of **4** was further supported the above observations. The position of carbonyl group located at either ring D or E could tentatively be assigned to C-22 position on the basis of the biogenetic consideration. In view of the above findings, the structure of **4** could be assigned as 22-oxo-3β,24-dihydroxy-olean-12-ene (soyasapogenol E)[11]. All the physical and spectral data were identical with those appeared in literature[8,11,18] and direct comparison with an authentic sample established its identity. This is the first report of the isolation of soyasapogenols B and E from the genus Melilotus. Although soyasapogenols have been isolated from many plant sources[7~9,11~29], it is of interest to note that soyasapogenols were only found in Leguminosae.

EXPERIMENTAL METHODS

Mps were determined on a Mitamura-Riken apparatus and are uncorrected. IR spectra were recorded in KBr disc in Perkin-Elmer 283B spectrophotometer. Optical rotations were recorded on a Rudolph Autopol III automatic polarimeter. ^{1}H-NMR spectra were run on a Varian FT-80A instrument operating at 80 MHz and chemical shift values are quoted in ppm down-field from TMS as internal standard. Mass spectra were obtained on a Hewlett-Packard 5985B GC/MS system equipped with a direct inlet system and operating at 70 eV. UV spectra were run with Gilford System 2600 spectrophotometer.

Plant Material

The aerial parts of *M. officinalis* (Herba Meliloti conc., DAB 6) were purchased from Apotheke im Stathaus, Bonn, West Germany and identified by Prof. H.J. Chi. A voucher specimen is on deposit at Natural Products Research Institute.

Extraction and fractionation

The chopped material (0.8kg) was refluxed with MeOH for 3 hr 5 times and concentrated *in vacuo*. The MeOH extract was partitioned between hexane and 10% aqueous MeOH to give hexane fraction (21.5g). The aqueous layer was partitioned with $CHCl_3$ and then BuOH to yield $CHCl_3$ (6.2g) and BuOH (48.8g) fractions, respectively.

Acid hydrolysis of the BuOH fraction

A solution of a portion of the BuOH fraction in 5% HCl-60% dioxane was refluxed for 5 hr. The reaction mixture was concentrated to the half volume *in vacuo* and diluted with water to afford

Table I. The Chemical Shifts of the Methyl Groups for 3β,22β,24-triacetoxyolean-12-ene.

Substituents	C-CH_3							
	C-23	C-24	C-25	C-26	C-27	C-28	C-29	C-30
3β-OAc [10]	0.86	0.86	0.96	0.96	1.13	0.83	0.86	0.86
22β-OAc [10]	0.01	–	0.02	0.02	0.02	0.15	−0.04	0.01
24-OAc [10]	0.16	–	−0.01	−0.01	−0.01	–	−0.01	0
Cald	1.03	–	0.97	0.97	1.14	0.98	0.81	0.87
found	1.02	–	0.97	0.97	1.14	0.97	0.81	0.89

precipitate. The precipitate was filtered and dried to yield darkbrown powder. Repeated flash column chromatography of the powder on silica gel (hexane-ethylacetate=8 : 5) followed by recrystallization of the resulting major subfraction from MeOH furnished needles of **1** (400 mg), **2** (10 mg), **3** (200 mg) and **4** (10 mg).

***Compound* 1**

mp 67-8° [Lit.[30] mp 67-8°] IR ν_{max}^{KBr} cm^{-1} 1705(α,β-unsaturated C=O), 1607, 1562, 1455 (C=C). UV λ_{max}^{MeOH} nm (log ε) 275(3.71), 311 (3.42). MS, *m/z* (rel. int.) 146(M^+, 53.0), 118(M^+-CO, 100), 90(M^+-2CO, 43.5), 89[M^+-(2CO+H), 45.6], 63(36.7), 51(22.8), 50(25.6). NMR($CDCl_3$, TMS) δ 6.39(1H, d, J=9.5Hz, H-3), 7.65(1H, d, J=9.5Hz, H-4), 7.25-7.54(4H, m, H-5, 6, 7 and 8).

***Compound* 2**

mp 276-8° [Lit.[31] mp 276°] IR ν_{max}^{KBr} cm^{-1} 3400(OH), 1657(α,β-unsaturated C=O), 1609, 1502(C=C). UV λ_{max}^{MeOH} nm(log ε) 267(3.72), 326(3.50), 367(3.78) ; λ_{max}^{MeONa} 279(3.80), 321(3.54), 415(3.84) ; λ_{max}^{NaOAc} 274(3.79), 307 (3.55), 380(3.74) ; $\lambda_{max}^{AlCl_3}$ 270(3.80), 305(3.30), 352(3.42), 426(3.85) ; $\lambda_{max}^{AlCl_3+HCl}$ 261(3.72), 271(3.77), 305(3.31), 351(3.47), 425(3.83) ; $\lambda_{max}^{NaOAc+H_3BO_3}$ 268(3.73), 324(3.51), 368(3.79). MS, *m/z* (rel. int.) 286(M^+, 77.9), 285(M^+-H, 27.9), 257(M^+-HCO, 13.7), 229(257-CO, 15.5), 184 (5.8), 153(AH^+, 17.7), 143(19.9), 136(14.2), 129(18.1), 121(B_2^+, 56.2), 108(15.9), 105 (15.0), 93(B_2^+-CO, 21.2). NMR(DMSO-d_6, TMS) δ 6.17(1H, d, J=2Hz, H-6), 6.42(1H, d, J=2 Hz, H-8), 6.87(2H, d, J=8.9Hz, H-3′, 5′), 7.98(2H, d, J=8.9Hz, H-2′, 6′), 12.49(1H, brs, H-5).

***Compound* 3**

mp 259-260° , $[\alpha]_D^{22}$+86.7° (c 0.12, MeOH) [Lit.[9] mp 259-260° , $[\alpha]_D$ +90°] IR ν_{max}^{KBr} cm^{-1} 3400(OH), 1650, 810, 800(C=C). MS, *m/z* (rel. int.) 458(M^+, 1.6), 443(M^+-CH_3, 0.2), 440(M^+-H_2O, 0.6), 425[M^+-(H_2O+CH_3), 0.5], 422(M^+-$2H_2O$, 0.2), 412[M^+-(CH_3+CH_2OH), 0.2], 409 [M^+-(H_2O+CH_2OH), 0.4], 391[M^+-(2H_2O+CH_2OH), 0.1], 234(D/E rings, 100), 224(A/B rings, 10.4), 223(3.5), 219(234-CH_3, 45.6), 216(234-H_2O, 12.7), 206(224-H_2O, 10.4), 201(219-H_2O, 12.8), 175(206-CH_2OH, 40.1), 135(21.5), 133(21.5).

NMR($CDCl_3$, TMS) δ 0.86(3H, s, CH_3), 0.90(6H, s, 2x CH_3), 0.94(3H, s, CH_3), 1.03(3H, s, CH_3), 1.11(3H, s, CH_3), 1.24(3H, s, CH_3), 3.33 and 4.20(1H each, d, J=11.1Hz, H-24), 3.35(2H, m, H-3 and H-22), 5.24(1H, t, J=3Hz, H-12).

***Acetylation of* 3**

A sample of **3** (20 mg) was treated with pyridine and acetic anhydride (1 m*l* each) and allowed to stand at room temperature overnight. After usual workup, the reaction product was crystallized from MeOH to yield **5** as fine needles. mp 180-1° , $[\alpha]_D^{22}$+80.8° (c 0.28, $CHCl_3$) [Lit.[9] mp 179-180° , $[\alpha]_D$ +78°] IR ν_{max}^{KBr} cm^{-1} 1725, 1245, 1232 (OAc). MS, *m/z* (rel. int.) 584(M^+, 2.5), 525(M^+-CH_3COO, 0.6), 524(M^+-HOAc, 1.3), 509[M^+-(CH_3+HOAc), 1.4], 464(M^+-2HOAc, 0.4), 451[M^+-(HOAc+CH_2OAc), 0.3], 391[M^+-(2HOAc+CH_2OAc), 0.3], 307(0.3), 276(D/E rings. 11.1), 261(276-CH_3, 0.4), 247(307-HOAc, 1.4), 216(276-HOAc, 100), 201[276-(HOAc+CH_3), 32.0], 187(307-2HOAc, 17.6), 174[307-(HOAc+CH_2OAc), 14.8]. NMR($CDCl_3$, TMS) δ 0.81(3H, s, CH_3), 0.89(3H, s, CH_3), 0.97(9H, s, 3×CH_3), 1.02(3H, s, CH_3), 1.14(3H, s, CH_3), 2.02 (6H, s, 2×OAc), 2.05(3H, s, OAc), 4.12 and 4.34 (1H each, d, J=11.7Hz, H-24), 4.59(1H, t, J=7.4Hz, H-3), 4.64(1H, t, J=3.6Hz, H-22), 5.26(1H, t, J=3Hz, H-12).

***Compound* 4**

mp 246-7° , $[\alpha]_D^{22}$+32.2° (c 0.2, MeOH) [Lit.[18] mp 249-250° , $[\alpha]$ +34°]. IR ν_{max}^{KBr} cm^{-1} 3250-3400(OH), 1706(carbonyl), 1650, 830, 806 (C=C). MS, *m/z* (rel. int.) 456(M^+, 1.6), 232(D/E rings, 100), 224(A/B rings, 16.5), 217(232-CH_3, 25.1), 206(224-H_2O, 21.3), 204(232-CO, 10.6), 203(232-HCO, 18.0), 189[232-(CH_3+CO), 21.9], 175[224-(H_2O+CH_2OH), 64.7]. NMR($CDCl_3$, TMS) δ 0.86(3H, s, CH_3), 0.90(3H, s, CH_3), 0.94 (3H, s, CH_3), 0.99(6H, s, 2×CH_3), 1.22(3H, s, CH_3), 1.25(3H, s, CH_3), 3.33 and 4.21(1H each, d, J=11Hz, H-24), 3.42(1H, t, J=8Hz, H-3), 5.30(1H, t, J=3Hz, H-12).

ACKNOWLEGEMENTS

This investigation was supported in part by Ministry of Education to SSK. The authors thank Professors T. Nohara, Kumamoto Univ., Japan, and M. Kaneda, Hiroshima Univ., Japan, for supplying authentic samples of soyasapogenols B and E, respectively.

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