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MELILOTIGENIN, A NEW SAPOGENIN FROM *MELILOTUS OFFICINALIS*

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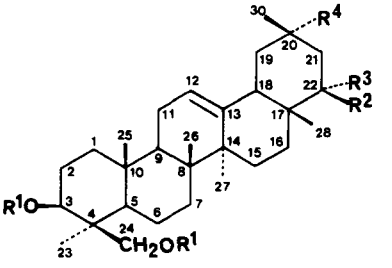
The dried leaves and flowers of *Melilotus officinalis* Lam. (Leguminosae) have been used in Europe as a treatment for arthritis, brachialgia, bronchitis, hemorrhoids, and rheumatism (1). A previous chemical investigation of the plant resulted in the isolation and characterization of soyasapogenols B [**1**] and E [**2**] together with coumarin, kaempferol, and quercetin (2). Further study on this drug has led to the isolation and structure elucidation of a new sapogenin **5**, characterized as its methyl ester **3**.

The ester **3**, mp 264–265°, gave a positive result in the Liebermann-Burchard test. In its ir spectrum, **3** showed characteristic signals at 3480 (OH), 1731 (ester), 1709 (carbonyl), 1650, and 810 cm^{-1} (C=C). Compound **3** gave a diacetate **4** upon acetylation. On hydrolysis with 10% KOH in MeOH, **3** afforded a desmethylated product **5**, which was converted to **3** by treatment with CH_2N_2 or methanolic HCl.

The nmr data of **3**, **4**, and **5** indicated that **3** was an olean-12-ene having a primary alcohol, a secondary alcohol, a carbomethoxy, and a ketone. The mass spectrum of **3** exhibited a molecular ion peak at *m/z* 500 and significant fragment peaks resulting from retro-Diels-Alder cleavage of ring C at *m/z* 276 and *m/z* 224 indicating that two hydroxyl groups were located at rings A and B and the carbomethoxy and ketone groups at rings D and E (3). Appearance of a hydroxymethyl AB quartet at a somewhat lower field (δ 3.78) than the known chemical shift value of an equatorially oriented hydroxymethyl group at the C-4 position (4) as well as comparison with the ^{1}H-nmr data of soyasapogenols B [**1**] and E [**2**] permitted the assignment of the 3β- and 24-hydroxy functionalities.



1 $R^1=R^3=H$, $R^2=OH$, $R^4=Me$
2 $R^1=H$, $R^2=R^3=O$, $R^4=Me$
3 $R^1=H$, $R^2=R^3=O$, $R^4=COOMe$
4 $R^1=Ac$, $R^2=R^3=O$, $R^4=COOMe$
5 $R^1=H$, $R^2=R^3=O$, $R^4=COOH$
6 $R^1=Ac$, $R^2=OAc$, $R^3=H$, $R^4=Me$

The point of attachment of the carbomethoxy group was allocated to the C-20 position by the observation of much smaller peaks at *m/z* 217 (7.7%) and 216 (5%) corresponding to the release of AcO or HOAc from an RDA fragment composed of rings D and E (3). The position of the ketone group could be tentatively assigned to a C-16 or C-22 position, because **3** did not display the ready decarboxylation of a β-keto ester during the alkaline hydrolysis nor did **3** undergo the McLafferty rearrangement in its mass spectrum, thus excluding a carbonylation at either C-15, C-19, or C-21 (3,5).

With the aid of a homonuclear 2D-nmr spectrum, the configuration of the carbomethoxy group at C-20 and the position of the ketone group could be assigned. The allylic H-18 proton at δ 2.37 was shown to be correlated with H-19_{ax} at δ 2.48 and a hidden H-19_{eq} signal at δ 1.70. A doublet at δ 2.99 and a single-proton double doublet centered at δ 2.30 were assigned to H-21_{ax} and H-21_{eq}, respectively. The presence of a W-type long-range coupling between

$H\text{-}19_{eq}$ and one of the methylene protons ($H\text{-}21_{eq}$) adjacent to the ketone group established the site of oxidation at C-22. Cross peaks arising from a W-type long-range coupling between $H\text{-}19_{ax}$ and the singlet at δ 1.16 and an analogous coupling between $H\text{-}21_{ax}$ and the same singlet that is assignable to the methyl group at C-20 permitted the assignment of an equatorial (C-29) disposition for the carbomethoxy group (6).

Confirmation of the assignment of the structure of **3** as methyl 3β,24-dihydroxy-22-oxo-olean-12-en-29-oate was obtained by the comparison of the ^{13}C-nmr chemical shifts of **4** and **5** with those for known soyasapogenol B acetate [**6**] and soyasapogenol E [**2**] (Table 1).

The characteristic chemical shift changes for the ring E carbons occurring on transformation of **2** into **4** are in close agreement with those of the reported C-29 carbomethoxylation-induced shift values (7,8). Because acid hydrolysis of the *n*-BuOH-soluble fraction in dioxane did not produce **3** but **5**, and **5** was easily methylated by treatment with methanolic HCl, it is suggested that **3** was formed during the acid hydrolysis in MeOH solution and that the acid **5** is a genuine sapogenin. This appears to be the first recorded instance of the occurrence of **5** in nature, and it is named melilotigenin.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—Melting points were determined on a Mitamura-Riken apparatus and are uncorrected. Ir spectra were measured on a Perkin-Elmer 283B ir spectrometer. Low and high resolution ^1H-nmr spectra were recorded in $CDCl_3$ solution with a Varian FT-80A (80 MHz) and a Nicolet NT-360 instrument (360 MHz), respectively, using TMS as an internal reference. ^{13}C-nmr spectra were obtained on either a Varian FT-80A (20 MHz), a JEOL FX-100 (25 MHz), or a Bruker AM-200 (50.3 MHz) spectrometer using TMS as an internal standard. Mass spectra were determined on a Hewlett-Packard 5985B gc/ms System at 70 eV

TABLE 1. ^{13}C-nmr Chemical Shifts (in ppm) of Compounds **2** (50.3 MHz), **4** (25 MHz), **5** (50.3 MHz), and **6** (20 MHz).[a]

Carbon	Compound				Carbon	Compound			
	2	**4**	**5**[b]	**6**[c]		**2**	**4**	**5**	**6**
1	38.43	38.45	38.79	38.44	20	34.04	47.98	47.06	30.41
2	27.67	23.56	27.62	23.63	21	50.88	45.53	44.42	38.54
3	80.88	80.10	80.61	80.21	22	216.69	214.89	217.36	78.39
4	42.86	44.24	42.71	41.76	23	22.41	22.53	22.74	22.53
5	55.93	55.91	56.25	56.03	24	64.49	65.42	64.74	65.34
6	18.44	19.29	18.62	19.35	25	16.08	15.46	16.33	15.42
7	32.99	33.04	33.33	33.06	26	16.74	16.57	16.98	16.67
8	39.70	41.02	41.24	39.93	27	25.38	25.31	25.63	25.96
9	47.69	47.57	47.06	47.72	28	20.57	20.43	20.65	27.01
10	36.76	36.81	37.07	36.33	29	32.00	176.50	179.50	33.46
11	23.80	23.56	24.18	23.63	30	25.38	20.98	20.84	20.98
12	123.63	124.45	125.06	122.32	−OAc		170.60		170.25
13	141.65	140.68	141.06	143.87			170.98		170.35
14	41.90	41.69	42.15	41.15			21.13		170.73
15	25.16	24.96	25.40	25.96			21.13		20.70
16	27.19	27.16	27.62	27.01					20.98
17	39.70	39.65	40.12	36.82					21.19
18	47.69	46.49	46.05	44.63	−OMe		52.28		
19	46.74	40.82	40.12	46.05					

[a]Unless stated otherwise, spectra were recorded in $CDCl_3$ and assignments were made by DEPT spectra.

[b]In $CDCl_3 + CD_3OD$.

[c]Assignments were made by APT spectrum and by comparison with data from Fukunaga *et al.* (9).

using a direct inlet system. Elemental analyses were performed on a Perkin-Elmer 240C Elemental Analyzer. Optical rotations were obtained on a Rudolph Autopol III automatic polarimeter.

PLANT MATERIAL.—The dried leaves and flowers of *M. officinalis* (Herba Meliloti Conc., DAB 6) were purchased from Apotheker im Stathaus, Bonn, West Germany. A voucher specimen was placed in our Institute.

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

ACID HYDROLYSIS OF THE *n*-BuOH FRACTION.—A portion of the *n*-BuOH fraction (20 g) in 5% HCl/MeOH (500 ml) was refluxed for 5 h. The reaction mixture was concentrated to half volume in vacuo and diluted with H_2O to afford a brown precipitate. Repeated cc of the precipitate on Si gel [hexane-EtOAc (8:5)], followed by recrystallization from MeOH, furnished shining plates of **3** (50 mg), mp 264–265°, $[\alpha]^{15}D$ +41.5° (c = 0.07, $CHCl_3$); ir ν max (KBr) 3480, 2920, 1731, 1709, 1650, 1383, 1265, 1227, 1054, 1026, 835, 810 cm^{-1}; ^1H nmr (80 MHz, $CDCl_3$) δ 0.90, 0.93, 1.01, 1.15, 1.22, 1.25 (3H each, s, 6 × Me), 3.35, 4.21 (1H each, d, J = 11.1 Hz, H-24), 3.42 (1H, t, J = 8 Hz, H-3), 3.69 (3H, s, OMe), 5.33 (1H, t, J = 3.4 Hz, H-12); ms *m/z* $[M]^+$ 500 (1.1%), $[M-H_2O]^+$ 482 (0.3), $[M-CH_2OH]^+$ 469 (0.3), $[M-(H_2O+Me)]^+$ 467 (0.2), $[M-2H_2O]^+$ 464 (0.3), $[M-(H_2O+CH_2OH)]^+$ 451 (0.3), [D/E ring]$^+$ 276 (26.2), $[276-Me]^+$ 261 (4.2), $[276-CHO]^+$ 247 (9.5), $[276-MeOH]^+$ 244 (3.5), [A/B ring]$^+$ 224 (21.8), $[276-(HOAc+H)]^+$ 215 (15.2), 207 (15.3), $[224-H_2O]^+$ 206 (18.0), 187 (20.7), 185 (10.0), $[224-(H_2O+CH_2OH)]^+$ 175 (59.8), 173 (20.4), 159 (19.4), 147 (25.1), 145 (21.8), 143 (24.5), 133 (37.0), 114 (100), 105 (40.9). Calcd for $C_{31}H_{48}O_5$: C 74.34, H 9.67. Found: C 74.28, H 9.53%.

The acid hydrolysis in 5% HCl/60% dioxane for 5 h furnished the free acid **5** instead of the methyl ester **3**.

ACETYLATION OF **3**.—Compound **3** (10 mg) was treated with Ac_2O/pyridine (0.5 ml each) at room temperature overnight. Work-up in the usual manner followed by recrystallization from MeOH afforded **4** as plates, mp 237–238°, $[\alpha]^{26}D$ +140° (c = 0.04, $CHCl_3$); ir ν max (KBr) 1736 (ester), 1709 (C=O), 1650, 1380, 1256, 1245, 1225, 1045, 812 cm^{-1}; ^1H nmr (360 MHz, $CDCl_3$) δ 0.96, 0.99, 1.02, 1.03, 1.16, 1.22 (3H each, s, 6 × Me), 2.05, 2.07 (3H each, s, 2 × OAc), 2.30 (1H, dd, J = 14.8, 2.8 Hz, H-21_{eq}), 2.37 (1H, br dd, J = 13.2, 3.5 Hz, H-18), 2.48 (1H, t, J = 13.2 Hz, H-19_{ax}), 2.99 (1H, d, J = 14.8 Hz, H-21_{ax}), 3.70 (3H, s, OMe), 4.15, 4.37 (1H each, d, J = 11.5 Hz, H-24), 4.58 (1H, dd, J = 10.2, 5.6 Hz, H-3), 5.33 (1H, t, J = 3.5 Hz, H-12); ms *m/z* $[M]^+$ 584 (0.8), $[M-HOAc]^+$ 524 (1.1), $[M-2HOAc]^+$ 464 (1.3), $[M-(2HOAc+Me)]^+$ 449 (1.7), $[M-(2HOAc+OAc)]^+$ 405 (0.4), [A/B ring]$^+$ 307 (1.1), [D/E ring]$^+$ 276 (100), $[276-Me]^+$ 261 (14.2), 248 (22.7), $[307-HOAc]^+$ 247 (29.2), 229 (6.7), $[276-OAc]^+$ 217 (10.4), $[276-(HOAc+H)]^+$ 215 (32.1), 201 (13.7), 199 (24.3), 189 (31.1), $[307-2HOAc]^+$ 187 (37.0), 175 (13.5), 173 (23.4), 159 (20.7), 147 (20.1), 133 (34.2), 119 (33.1), 114 (91.2), 105 (24.4).

ALKALINE HYDROLYSIS OF **3**.—Compound **3** (20 mg) was refluxed with 10% KOH/MeOH (20 ml) for 3 h. The reaction mixture was evaporated in vacuo and acidified with HCl to which crushed ice was added and extracted with Et_2O. Work-up in the usual way followed by crystallization from MeOH yielded **5** as shining plates, mp 318–319°, $[\alpha]^{18}D$ +57.1 (c = 0.04, MeOH); ir ν max (KBr) 3500, 3390 (OH), 2840, 1725 (C=O), 1700 (acid), 1620, 1380, 1220, 1210, 1048, 820, 810 cm^{-1}; ^1H nmr (80 MHz, $CDCl_3$) δ 0.89, 0.93, 1.01, 1.18, 1.22, 1.25 (3H each, s, 6 × Me), 3.35, 4.21 (1H each, d, J = 11.2 Hz, H-24), 3.42 (1H, m, H-3), 5.34 (1H, t, J = 3.4 Hz, H-12); ms *m/z* $[M]^+$ 486 (0.8), 394 (1.7), 227 (8.7), [D/E ring]$^+$ 262 (51.2), $[262-Me]^+$ 247 (4.6), $[262-CHO]^+$ 233 (10.3), 225 (10.7), [A/B ring]$^+$ 224 (19.6), $[262-COOH]^+$ 217 (8.3), 215 (10.1), $[224-H_2O]^+$ 206 (22.3), 204 (26.2), 199 (17.9), $[224-CH_2OH]^+$ 193 (5.3), 189 (12.8), 187 (20.6), $[224-(H_2O+CH_2OH)]$ 175 (74.4), 173 (21.0), 159 (17.5), 149 (29.3), 147 (23.4), 135 (36.6), 119 (38.7), 109 (22.2), 107 (31.5), 105 (39.6), 95 (29.0), 91 (100). Compound **5** was converted to a methyl ester **3** by ethereal CH_2N_2 methylation.

METHYLATION OF **5**.—Compound **5** (mg) was refluxed with 5% HCl/MeOH (5 ml), and the reaction mixture was monitored by tlc. After 1.5 h reflux, **5** was completely methylated to yield **3**. Usual work-up afforded **3** as plates, mp 264–265°.

ACKNOWLEDGMENTS

High resolution ^1H-nmr spectra and ^{13}C-nmr spectra of **4** were measured at the Suntory Institute for Bioorganic Research, Osaka, Japan.

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