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J. Nat. Prod., **1992**, 55 (9), 1339-1342• DOI: 10.1021/np50087a031 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 9, pp. 1339-1342, September 1992

SOYASAPONIN VI, A NEW MALTOL CONJUGATE FROM ALFALFA AND SOYBEAN

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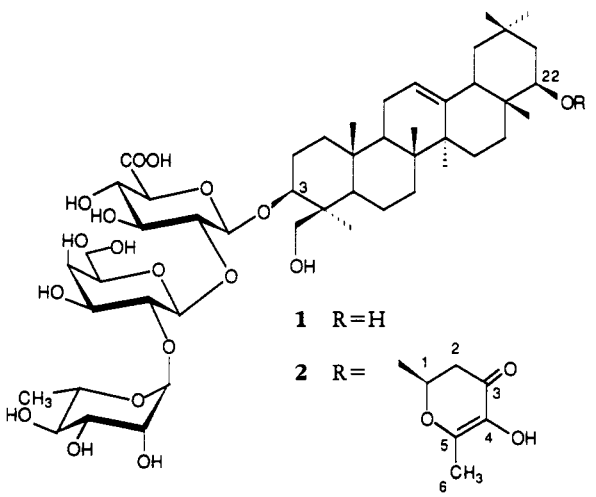
ABSTRACT.—Soyasaponin VI [**2**], a new O-22 maltol conjugate of soyasaponin I, has been isolated from alfalfa seeds and soybean. It is a labile compound and a possible precursor of soyasaponin I.

A major and often ignored problem in natural products chemistry is the determination of the origin, natural or artificial, of isolated material. Previously, we reported the isolation of soyasaponin I [**1**] (1,2) from the seeds of alfalfa (3) (lucerne, *Medicago sativa* L.) (Leguminosae). On examination of the saponins from germinating alfalfa seeds, we isolated, along with **1**, a new and unstable saponin **2**, for which we propose the name of soyasaponin VI and which might be the natural precursor of **1**. We describe here the isolation and structural elucidation of **2**.

After preliminary defatting of the plant (alfalfa or soybean), saponins were extracted with aqueous MeOH and partitioned between *n*-BuOH and H_2O. Evaporation of the *n*-BuOH phase gave a residue which was dissolved in MeOH and precipitated with Et_2O. Final purification was obtained through a combination of cc and tlc. The fastest running band is soyasaponin VI (approximate yield 0.1%); the slowest moving band corresponds to soyasaponin I, which was identified by direct comparison with an authentic sample kindly provided by Prof. I. Kitagawa (yield ca. 0.07%). Neutrality of the pH during all the extraction phase is of paramount importance for the success of the isolation, and steps such as filtration on ion exchange resins must be avoided.

The presence of a new compound in the saponin fraction was suspected from the observation in the ^{1}H-nmr spectra of crude fractions of an AMX system with X in the olefinic protons range and AM in the 2.5–3 ppm area. Purification was thus monitored by ^{1}H nmr, and prepara-



tive tlc finally offered **2** as white amorphous powder melting at 280° (dec) and with $[\alpha]_D$ 1° (MeOH, c=0.13). Compound **2** stained dark pink on tlc after H_2SO_4 spraying and heating. Solutions of **2** slowly decomposed into soyasaponin I [**1**].

The structural elucidation of **2** was based on spectroscopic evidence, on 1D and 2D nmr and mass spectrometry, and on the fact that **2** decomposed into **1**. The ^{1}H-nmr spectrum of **2** (CD_3OD, 300 MHz, Table 1) was assigned by means of COSY, HMQC, and HMBC experiments. For sensitivity reasons, a ^{13}C-nmr spectrum could not be directly measured, but ^{13}C resonances for 53 of the 54 carbon atoms were obtained by the ^{1}H detected-^{13}C modulated HMBC and HMQC experiments (Table 1). All the signals corresponding to the four components of soyasaponin I (soyasapogenol B, glucuronic acid, galactose, and rhamnose) were found at chemical shifts similar to the ones observed in **1** (4,5). Extra proton signals were observed at δ 5.39 (t, 1H, J=3.3 Hz), 2.92 (dd, I H, J=3.3, 16.5 Hz), 2.51 (dd, 1H, J=3.3, 16.5 Hz), and 2 ppm (s, 3H). Extra indirectly detected carbons appeared at δ 190.5 (C), 158.5 (C), 137 (C), 99 (CH), 43.5 (CH_2). and 16.7 (Me). The large value for the geminal coupling of the methylene protons (−16.5 Hz) pointed to a position α to a carbonyl. The δ value for this atom (190.5 ppm) is intermediate between those of

TABLE 1. ^{1}H and ^{13}C nmr for Compound **2** (CD_3OD; 300 and 75 MHz, respectively).

Position	δC	δH	Position	δC	δH (m, J)
Genin			Glucuronic acid		
1	41.5	—	1	106.5	4.47 (d, 7.5)
2	28.2	1.99	2	78.1	3.78 (dd, 9–7.5)
3	94.5	3.4	3	79.2	3.59 (t, 9)
4	47	—	4	74.9	3.46 (t, 9)
5	58.6	—	5	77.9	3.72 (t, 9)
6	nd	—	6	nd	—
7	35.6	—			
8	42.5	—	Galactose		
9	50.3	—	1	103.5	4.86 (d, 7)
10	39	—	2	79.2	3.64 (dd, 7–9.6)
11	25.8	—	3	77.4	3.54 (dd, 3.4–9.6)
12	125	5.2	4	72.5	3.73 (m)
13	147.5	—	5	77.6	3.49 (m)
14	45	—	6	63.3	3.68–3.8 (m)
15	28.4	—			
16	30	—	Rhamnose		
17	40	—	1	103	5.13 (d, 1.5)
18	47.5	2.09	2	73.2	3.91 (dd, 1.5–3.1)
19	48.7	—	3	73.3	3.71 (dd, 3.1–9.5)
20	33.5	—	4	75.2	3.39 (t, 9.5)
21	39	—	5	70.6	4.08 (dq, 9.5–6.7)
22	85	3.47	6	20	1.26 (d, 6.7)
23	24.5	1.25			
24	66	3.21–4.13	Dihydromaltol		
25	17.3	0.89	1	99	5.39 (t, 3.3)
26	18.7	0.98	2	43.5	2.51–2.92 (dd, 3.3–16.5)
27	27.6	1.16	3	190.5	—
28	22.3	0.83	4	137	—
29	34.2	0.9	5	158.5	—
30	29.7	0.98	6	16.7	2

ketone (200–210 ppm) and ester (170–180 ppm) carbonyls and suggests a vinylogous ester function. On such a unit, the α and β carbon atoms are straightforwardly placed at 137 and 158.5 ppm, respectively. Following the HMBC experiment (two- or three-bond connectivities), the methyl, methylene, and methine are connected to the three carbon atoms of the vinylogous ester to form a γ-pyrone ring. A tertiary hydroxy group is placed on C-4 to account for the lack of a proton and for the chemical shifts of C-3 and of C-5. The dihydromaltol unit is placed on O-22 to account for the deshielding of C-22 (85 ppm in **2** vs. 78 ppm in **1**), for $^3J_{C-H}$ correlations (H-1 of maltol to C-22 of soyasapogenol), and for H-H transfers of magnetization (between H-1 of maltol and H-22 of soyasapogenol).

The structure of **2** was confirmed by ms. Negative fabms showed $[M-H]^-$ ion at 1067 corresponding to $C_{54}H_{84}O_{21}$. Positive fabms showed a quasi molecular ion at 1113 $[M+2Na-H]^+$ and a fragment corresponding to loss of maltol (987).

It is not difficult to imagine mechanisms by which **2** is split into **1** and maltol under either basic or acid conditions. The converse reaction, however, is more difficult to run and we have not been able so far to prepare maltol conjugates of moderately hindered alcohols from maltol.

Despite its lability, **2** exists as one isomer at the anomeric carbon, whose absolute configuration is proposed as depicted in Figure 1, on the basis of CD. The CD spectrum of **2**, recorded in MeOH, shows a strong negative Cotton effect corresponding to the uv absorption maximum of the enone chromophore

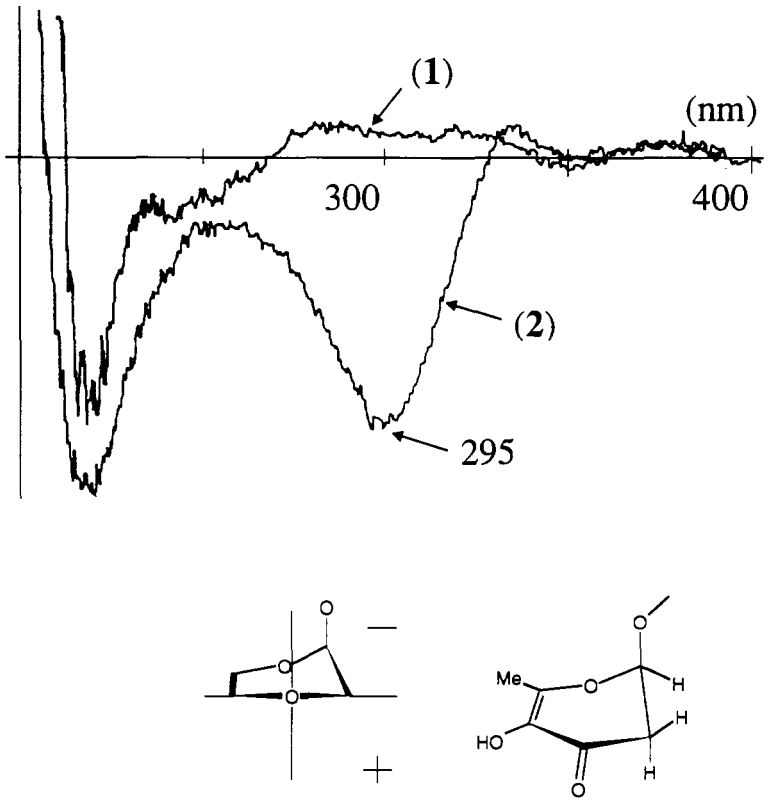


FIGURE 1. Cd spectra of soyasaponins I [**1**] and VI [**2**] and views of the dihydropyran ring from the plane of the carbonyl and from above the mean plane of the ring.

at $\lambda=295$ nm. The ^{1}H-nmr spectrum of **2**, which was also recorded in MeOH, shows that in this solvent, the dihydromaltol part of the molecule adopts a rigid conformation with the anomeric oxygen in an axial position due to stereoelectronic effects. Even though the octant rule does not strictly apply to conjugated ketones (6), the negative Cotton effect may be explained by a contribution from a substituent in the right upper rear octant, the 1-axial oxygen in the half chair drawn below. Work is in progress to further substantiate this hypothesis.

Soyasaponin VI was first isolated from alfalfa sprouts, and it was thought that this substance was produced during germination. Another study was then conducted on dormant seeds, and compounds **1** and **2** were both detected. Evaluation of the levels of **1** and **2** during germination requires a quick and reliable extraction procedure and hplc titration; this is not yet available due to difficulties in the detection of saponins by hplc. To verify whether or not maltol conjugates are only produced in alfalfa, seeds of soybean (*Glycine max* Merrill) (Leguminosae) were extracted and it was found that they contained soyasponin VI as well. Its presence had, until now, escaped attention.

The only natural product related to soyasaponin VI is the recently isolated γ-pyrone hoslundin (7), which is a C-maltol derivative.

EXPERIMENTAL

EXTRACTION.—Ground seeds and sprouts (350 g) of alfalfa were sequentially defatted with boiling hexane and $CHCl_3$ (1.5 liters each) and extracted with aqueous MeOH (4:1) (1.5 liters). The organic phase was concentrated in vacuo and the remaining aqueous phase extracted with *n*-BuOH saturated with H_2O (2×0.3 liter$+2\times0.15$ liter). The organic phases were evaporated, and the residue was dissolved in 0.2 liter of MeOH and diluted with 1 liter of Et_2O. The precipitate (4.5 g) was chromatographed on Si gel using mixtures of $CHCl_3$/MeOH/H_2O. Saponins **1** and **2** were obtained in pure form by preparative tlc [$CHCl_3$-MeOH-H_2O (12:8:1)]. Powdered soybean seeds (200 g) were sequentially extracted with hexane (2 liters) and MeOH (2 liters) under reflux. The MeOH extract was concentrated to 400 ml, and 2 liters of Me_2CO was added to precipitate the saponins (5.73 g). The operation was repeated with MeOH and Et_2O, and 2.42 g of precipitate was further obtained. The precipitates showed similar behaviors on tlc and were joined in a cellulose tubing for dialysis. After 3 days, the contents of the tube were lyophylized to yield 1.96 g of crude saponin mixture. An analytical sample was obtained by preparative tlc on reversed-phase Whatman PKC18F 1-mm plates [MeOH-0.5 M NaCl (75:25)].

SOYASAPONIN VI [**2**].—Mp 280° (dec); $[\alpha]_D$ 1° (MeOH, $c=0.13$); ir (KBr) cm^{-1} 3397, 1728, 1626, 1383, 1074, 1045; uv λ-max (MeOH) nm 204, 288; ^{1}H and ^{13}C nmr see Table 1.

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Received 2 April 1992