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Structure of Soyasapogenol B_1

Philip A. Ireland,* Stanley Z. Dziedzic, Michael G. B. Drew, and George A. Forsyth

The structure of soyasapogenol B_1, previously shown to be an artifact of hydrolysis, was elucidated by X-ray crystallography and confirmed by mass spectrometry as 3β,22β,24-trihydroxyolean-13(18)-ene.

We recently reported a method for the analysis of soybean sapogenins by normal-phase, high-performance liquid chromatography (HPLC) and evaporative light-scattering detection (Ireland and Dziedzic, 1985). Our work identified a previously unknown sapogenin, which we tentatively named soyasapogenol B_1 due to its mass spectrum and chromatographic behavior similar to that of soyasapogenol B (Ireland, 1987). Later work showed that soyasapogenol B_1, as well as soyasapogenols C–E, are artifacts of the acid hydrolysis of soybean saponins (Ireland and Dziedzic, 1986). This paper deals with the elucidation of the structure of soyasapogenol B_1 by X-ray crystallography and mass spectrometry.

EXPERIMENTAL SECTION

Isolation of Soyasapogenol B_1. Defatted soy flour was extracted with methanol for 30 h in a Soxhlet apparatus. After removal of the methanol under reduced pressure, the extract was hydrolyzed for 5 h by refluxing in a mixture of concentrated hydrochloric acid, water, and ethanol (2:1:3, v/v/v). The cooled hydrolysate was diluted with water, and the liberated sapogenins were extracted with diethyl ether. The ether extract was washed with 2% potassium hydroxide and water. The soyasapogenols were separated by column chromatography using silica gel and hexane/ethyl acetate (4:1, v/v) and subjected to preparative thin-layer chromatography (Ireland and Dziedzic, 1985). The thin-layer chromatography separation was repeated until pure soyasapogenol B_1 was obtained as determined by HPLC (Ireland and Dziedzic, 1985). Soyasapogenol B_1 was crystallized twice from methanol/water.

Mass Spectra. Mass spectra were recorded on a Kratos MS80RFA mass spectrometer with a Data General Desktop 30 and Kratos DS90 data system. An ionization potential of 70 eV, resolution 3000, 100-μA beam current, 3-s/decade scanning rate, and source temperature 250 °C were used.

X-ray Crystallography. Intensity data were collected on a Stoe Stadi 2 diffractometer using a variable-width ω-scan.

RESULTS AND DISCUSSION

Isolation of Soyasapogenol B_1. Hydrochloric acid in water/ethanol was used as the hydrolysis medium as it had

Department of Food Science and Technology, Food Studies Building, University of Reading, Whiteknights, Reading RG6 2AP, U.K. (P.A.I., S.Z.D.), and Department of Chemistry, University of Reading, Whiteknights, Reading RG6 2AD, U.K. (M.G.B.D., G.A.F.).
* To whom correspondence should be addressed.

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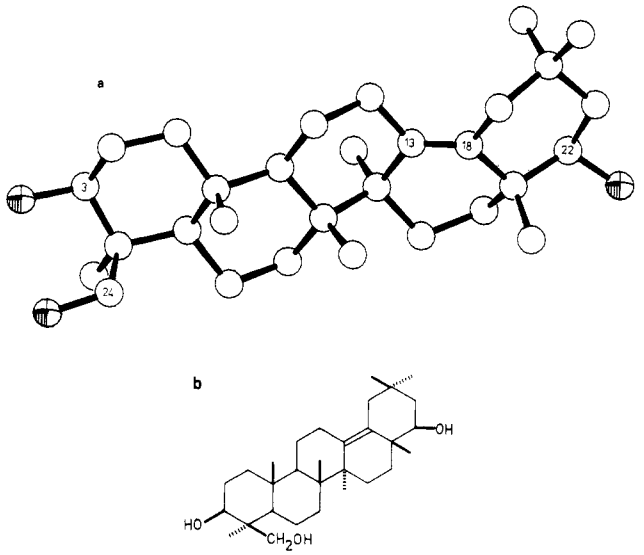

Figure 1. Structure of soyasapogenol B_1: (a) computer graphic; (b) line drawing.

Table I. Mass Spectra of Soyasapogenols B and B_1

	soyasapogenol intensity,[b] %		
m/z[a]	B[c]	B	B_1
458	4	4	38
235	18		
234	100	100	36
232	13		
224		8	
221			38
219	31	31	
216	18	10	
206	11	10	27
205			30
204			32
203			100
201		7	
187			28
176	25	25	
175	28	22	52

[a] Eight most intense ions plus molecular ion only are given. [b] Relative to base peak. [c] Yokota et al. (1982).

previously yielded a sapogenin fraction containing soyasapogenol B_1 but not soyasapogenol B (Ireland and Dziedzic, 1986). The absence of soyasapogenol B simplified the subsequent isolation of soyasapogenol B_1 from the sapogenin extract.

Crystal data: $C_{30}H_{50}O_3 \cdot H_2O$, M_r 458.7; monoclinic space group $P2_1$; $Z = 2$; $a = 12.829$ (8), $b = 7.156$ (8), $c = 14.664$ (7) Å; $\beta = 91.7$ (1)°. A total of 1407 independent reflections above background have been measured, and the structure, solved by direct methods, has been refined to $R = 0.10$. Carbon and oxygen atoms were refined anisotropically, and hydrogen atoms were refined isotropically. Positional parameters, molecular dimensions, and structure factor tables are available as supplementary material.

The structure of the molecule is shown in Figure 1. Of particular interest is the presence of a double bond at C(13)–C(18) of 1.272 (17) Å. The remaining dimensions in the structure are as expected and establish all other C–C and C–O bonds to be single. The water molecule (not shown in Figure 1) participates with the three hydroxide groups in intermolecular hydrogen bonding.

Mass Spectra. The crystal data indicate that soyasapogenol B_1 is a member of the olean-13(18)-ene class of triterpenes while soyasapogenols A–C and E have been shown to be olean-12-enes (Kitagawa et al., 1982). Olean-12-enes and olean-13(18)-enes should be distinguishable by the characteristic mass spectral fragmentations shown by these triterpenes (Budzikiewicz et al., 1963). A previous investigation of the mass spectra of these two compounds (Ireland, 1987) had shown few differences in the spectra, so the two sapogenins were reinvestigated under identical source and ionization conditions.

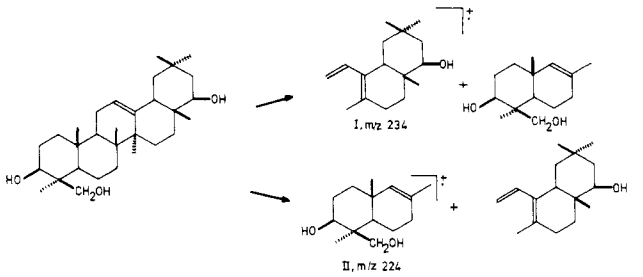

Figure 2. Reverse Diels–Alder fragmentation of soyasapogenol B.

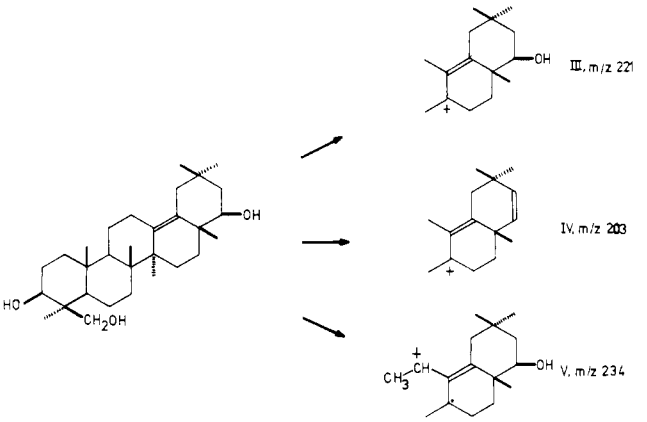

Figure 3. Major fragment ions in the mass spectrum of soyasapogenol B_1.

Table I gives the intensity of the eight most intense ions in the mass spectra of soyasapogenols B and B_1, together with the previously published data for soyasapogenol B (Yokota et al., 1982). Clearly, differences exist between the spectra of soyasapogenols B and B_1, while the spectrum of soyasapogenol B closely resembles the published data.

The dominant feature of the mass spectra of olean-12-enes has been observed to be a fragmentation equivalent to a reverse Diels–Alder reaction (Budzikiewicz et al., 1963), as shown in Figure 2. This process has been used as a diagnostic fragmentation in the identification of the soyasapogenols (Heftmann et al., 1979). As can been seen from Figure 2, two corresponding fragment ions, I and II, can be produced, but the diene fragment, ion I, is the more stable and predominates (Budzikiewicz et al., 1963), giving rise to the base peak in the mass spectra of the soyasapogenols (Berrang et al., 1974; Heftmann et al., 1979).

By analogy to the reported mass spectra of olean-13(18)-ene and olean-13(18)-en-3-one (Budzikiewicz et al., 1963), a base peak at m/z 221 would be expected for 3β,22β,24-trihydroxyolean-13(18)-ene (Figure 3). Although an ion (III) of this m/z value is a major component of the spectrum of soyasapogenol B_1, it is not the base peak. However, the base peak at m/z 203 could derive from the thermal elimination of water from soyasapogenol B_1 in the ion source of the mass spectrometer leading to an ion of m/z 203 (IV) by an analogous fragmentation process to the formation of ion III. Cleavage of the C(8)–C(14) and C(9)–C(11) bonds and shift of one hydrogen (Budzikiewicz et al., 1963) would produce an allylic cation of m/z 234 (V).

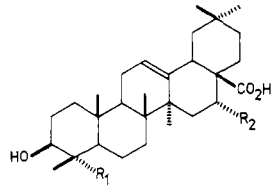

Figure 4. Olean-12-ene structures: quillaic acid, R_1 = CHO, R_2 = OH; echinocystic acid, R_1 = CH_3, R_2 = OH; gypsogenic acid, R_1 = CO_2H, R_2 = H.

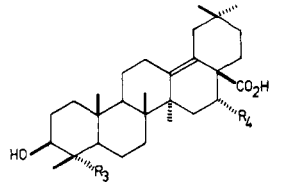

Figure 5. Olean-13(18)-ene structures: albigenic acid, R_3 = CH_3, R_4 = OH; 3β,16α-dihydroxyolean-13(18)-en-23-al-28-oic acid (olean-13(18)-ene isomer of quillaic acid), R_3 = CHO, R_4 = OH.

The presence of satellite ions around m/z 203 and 221 (not shown in Table I) probably results from fragmentations involving rearrangements and proton transfers as suggested by Budzikiewicz et al. (1963), but extensive mass spectral studies involving deuterium labeling would be required to rationalize these fragmentations. Of note is the relative stability of the molecular ion of soyasapogenol B_1 compared to soyasapogenol B. Clearly, the mass spectrum of soyasapogenol B_1, an olean-13(18)-ene, is consistent with the structure derived from X-ray crystallography and displays the expected differences to the mass spectrum of soyasapogenol B (an olean-12-ene).

CONCLUSIONS

Acid-catalyzed double-bond migration in triterpenes has been noted previously (Brownlie et al., 1956; Coates, 1967), and the isomerization of olean-12-enes to olean-13(18)-enes has been observed when saponins are subjected to hydrolysis using hydrochloric acid in aqueous ethanol (Kubota et al., 1969). The study of Kubota et al. (1969) revealed that quillaic acid and echinocystic acid (both olean-12-enes; Figure 4) are isomerized to the corresponding olean-13(18)-enes (Figure 5) on heating with hydrochloric acid in water/ethanol. However, this is not an isomerization displayed by all olean-12-enes as gypsogenic acid (another olean-12-ene; Figure 4) was recovered "almost quantitatively" after refluxing with hydrochloric acid in water/ethanol (Kubota et al., 1969).

We have not been able to identify the olean-13(18)-ene isomers of soyasapogenols A, C, or E in the aglycon fraction obtained when soya saponins are hydrolyzed in an aqueous acid environment. Clearly, structural features of the triterpene sapogenin affect its susceptibility to this type of isomerization.

It has also been shown that soyasapogenols C and E are artifacts formed on aqueous acid hydrolysis of soya saponins and that soyasapogenols A and B are the true aglycone. Further, unknown transformations of the soyasapogenols in aqueous acids have been indicated by the decrease in total sapogenin (i.e., soyasapogenols A, B, B_1, C, and E) yield from constant amounts of soya saponin on prolonged aqueous acid hydrolysis (Ireland and Dziedzic, 1986). All these isomerizations and transformations can be avoided by the use of sulfuric or hydrochloric acids in an anhydrous methanolic environment for the hydrolysis of saponins (Ireland and Dziedzic, 1986).

Registry No. Soyasapogenol B_1, 104033-83-2.

Supplementary Material Available: Listings of atomic coordinates, distances and angles, thermal parameters, and hydrogen atom coordinates (11 pages); table of observed and calculated structure factors (10 pages). Ordering information is given on any current masthead page.

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