Journal of Natural Products
Vol. 53, No. 2, pp. 298-302, Mar-Apr 1990

SAPOGENIN STRUCTURE: ANALYSIS OF THE ^{13}C- AND ^{1}H-NMR SPECTRA OF SOYASAPOGENOL B

ROBERT L. BAXTER,

Chemistry Department, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, UK

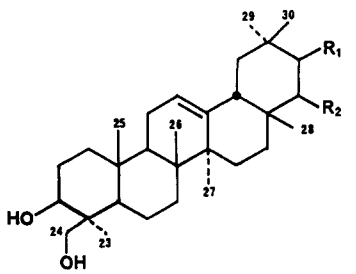
KEITH R. PRICE,* and G. ROGER FENWICK

AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA, UK

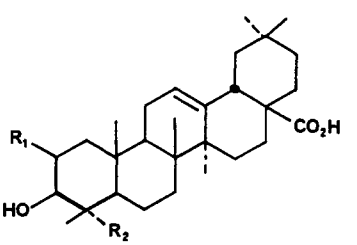
ABSTRACT.—The ^{13}C- and ^{1}H-nmr spectra of soyasapogenol B [**2**] have been assigned using ^{13}C DEPT, ^{13}C APT, ^{1}H-^{13}C COSY, ^{1}H-^{1}H COSY, and ^{1}H-^{1}H ROESY techniques. This sapogenin has been shown to be a principal triterpenoid aglycone in seeds of *Medicago sativa* (alfalfa) and *Pisum sativa* (pea).

The physiological properties of triterpenoid saponins in vegetable foodstocks are of significant medical and agricultural importance (1–3). While it is generally recognized that the biological effects of these compounds are linked to the substitution and configurations of the aglycone components, relatively little is known of the relationships between aglycone structure and biological activity (1–3). One particular point of confusion in the earlier literature on the structures of sapogenols has been the configuration of C-4 hydroxymethyl substituents in olean-12-ene triterpenoids isolated from different plant sources (4). The structures of soyasapogenols A [**1**], B [**2**], C [**3**], and E [**4**], isolated from soya (*Glycine max*) have recently been revised on the basis of the X-ray crystallography of the triacetate of **2** and shown to be hydroxylated at C-24 rather than C-23 (5). More recently, compounds suggested to be **1–4** have been identified in the sapogenol hydrolysate of alfalfa (*Medicago sativa*), a plant source which also produces the C-23 oxygenated aglycones, medicagenic acid [**5**], hederagenin [**6**], and bayogenin [**7**] (6). However, no attempt was made to assign the oxygen substitution to C-23 or C-24.

Certainly the unambiguous assignment of the configuration of C-4 hydroxymethyl substituents of olean-12-enes and related triterpenoid alcohols is complicated by similarities in both nmr and mass spectral characteristics. While numerous studies on the assignment of ^{13}C resonances by correlation of chemical shifts between closely related compounds and by single resonance ^{1}H-^{13}C decoupling experiments have been reported (7–12), neither complete ^{13}C-^{1}H correlations nor ^{1}H assignments are available. The increasing sophistication and dispersion of modern nmr spectrometers now permits detailed analysis of the ^{13}C and ^{1}H spectra of these molecules.



1 R_1=R_2=OH
2 R_1=H, R_2=OH
3 R_1,R_2= >C=C<
4 R_1=H, R_2= =O



5 R_1=OH, R_2=CO_2H
6 R_1=H, R_2=CH_2OH
7 R_1=OH, R_2=CH_2OH

RESULTS AND DISCUSSION

In this paper we describe the application of both single dimensional and two dimensional ^{13}C-^{1}H correlation techniques to assign the ^{13}C and ^{1}H resonances of **2** isolated from *Medicago sativa* seeds. Homonuclear 2D nmr methods have been used to identify both the principal ^{1}H-^{1}H couplings and through-space interactions, allowing unambiguous assignment of the ^{1}H resonances.

^{13}C-^{1}H CORRELATIONS.—Preliminary assignments of CH, CH_2, Me, and quaternary carbon resonances were made in the ^{13}C DEPT (13) and APT (14) experiments. Comparison of the observed chemical shifts with those reported for model compounds (7–12) and with calculated values enabled identification of the resonances of carbon atoms in unique environments. Thus, the olefinic methine carbon resonance at 122.2 ppm and quaternary carbon resonances at 143.8, 39.7, and 30.6 ppm could be assigned to C-12, C-13, C-8, and C-20, respectively (Table 1). The ^{13}C-^{1}H COSY-90 spectrum (15,16) of **2** (Figure 1) allows complete correlation of the protonated carbon resonances with the ^{1}H spectrum. While ^{1}H-^{1}H coupling data cannot be extracted with confidence from this type of spectrum, the resolution of the higher frequency components of the H-1 and H-7 methylene signals as apparent triplets (J = ca. 11 Hz) allows these to be assigned to axial hydrogens.

^{1}H-^{1}H-CONNECTIVITIES.—Analysis of the complex overlapping proton signals in the 0.8–2.1 ppm region of the proton spectrum was carried out using ^{1}H-^{1}H COSY (Figure 2). The assignment of C-ring protons, for example, was evident from cross

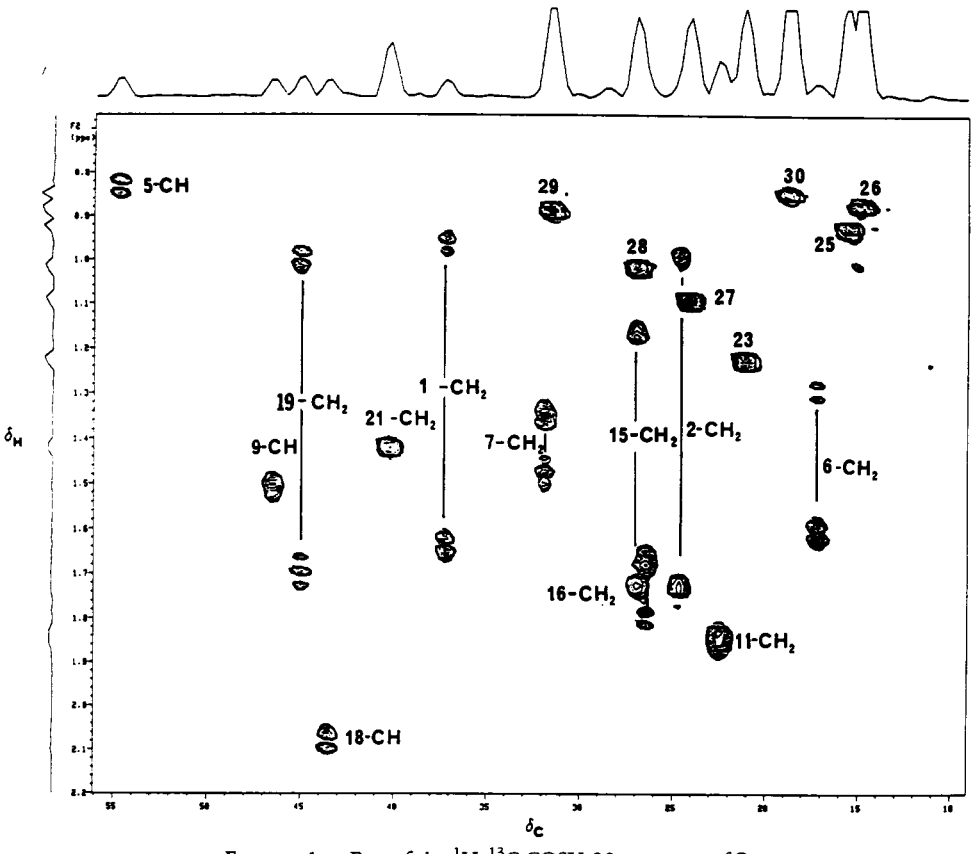


FIGURE 1. Part of the ^{1}H-^{13}C COSY-90 spectrum of **2**.

TABLE 1. ^{13}C and ^{1}H Assignments for Soyasapenol B [2].

Position	Chemical Shifts[a]		Position	Chemical Shifts	
	^{13}C	^{1}H[b]		^{13}C	^{1}H
1	38.4	0.96 eq, 1.65 ax	16	27.5	1.65 eq, 1.80 ax
2	25.6	0.98 eq, 1.72 ax	17	36.7[d]	—
3	76.6	3.42	18	44.8	2.08
4	42.1[c]	—	19	46.2	0.99 eq, 1.69 ax
5	55.9	0.83	20	30.6	—
6	18.5	1.30 eq, 1.60 ax	21	41.5	1.42 eq, 1.42 ax
7	33.1	1.32 eq, 1.46 ax	22	80.9	3.42
8	39.7	—	23	22.5	1.22
9	47.7	1.50	24	64.5	3.40, 4.20
10	37.4[d]	—	25	16.20	0.87
11	23.6	1.83 eq, 1.83 ax	26	16.17	0.92
12	122.2	5.22	27	25.5	1.08
13	143.8	—	28	28.2	1.01
14	42.8[c]	—	29	32.9	0.88
15	28.0	1.16 eq, 1.72 ax	30	20.9	0.84

[a]Ppm relative to TMS.
[b]^{1}H chemical shifts assigned on the basis of ^{13}C-^{1}H COSY-90 and ^{1}H-^{1}H COSY-45 experiments.
[c,d]Assignments with the same superscript may be interchanged.

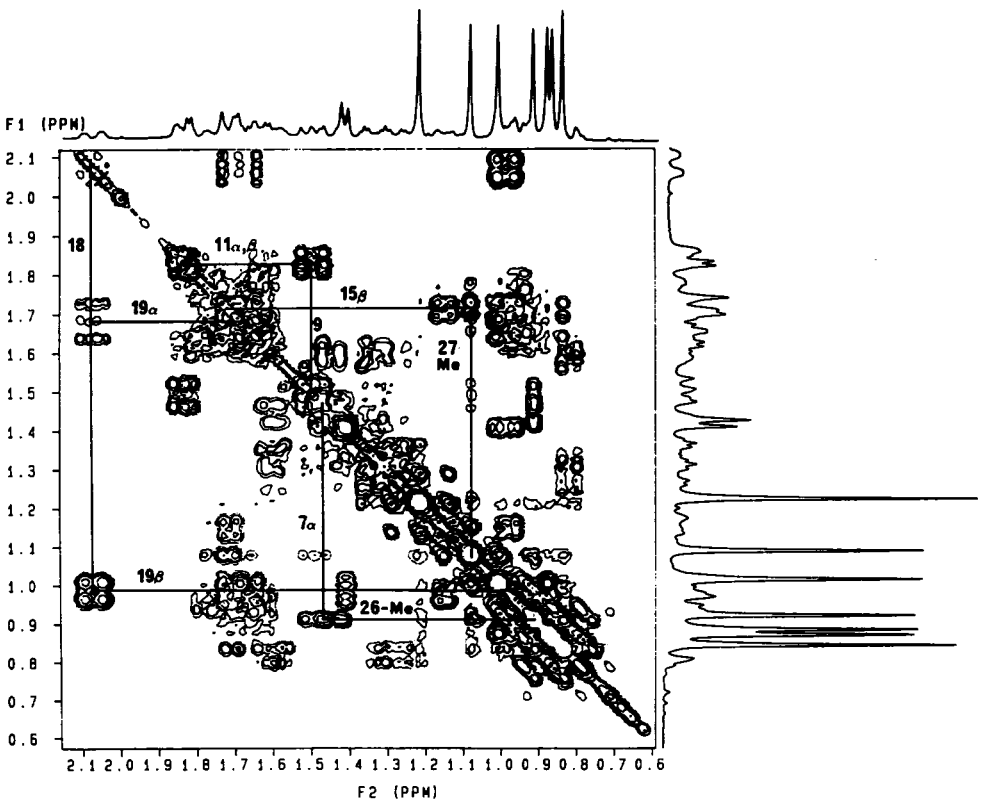


FIGURE 2. The 0.6–2.1 ppm region of the ^{1}H-^{1}H COSY-45 spectrum of **2** in $CDCl_3$. Representative cross peaks described in the text are indicated (300 MHz, spectral width 1463 Hz in F_1 and F_2, data set 512 points in both dimensions, dimension of transformation 1000 × 1000 Hz).

peaks corresponding to H-12 coupling to the CH_2-11 multiplet at 1.82 ppm which is also strongly coupled to the CH-9 resonance at 1.50 ppm. Ambiguities in the assignments of the C-6/C-7 and C-15/C-16 methylene protons could be resolved by observation of couplings between the H-7 axial proton and the Me-26 and between H-15ax and the Me-27.

While the resonances of the seven methyl groups in the molecule are well resolved in the ^{1}H nmr, their assignment on the basis of chemical shifts is suspect. Solvent and lanthanide shift effects have been exploited (10,17) to differentiate methyl groups in triterpenoids, but their application to polyfunctional molecules is fraught with difficulties in interpretation of results. The identification of through-space interactions provides a firmer basis for assignment. The 500 MHz ^{1}H-^{1}H ROESY-45 spectrum (18) of **2** showed cross peaks corresponding to the through-space interactions shown in Figure 3, enabling the methyl shift assignments shown in Table 1 to be made. Of particular note are the nOe's resulting from proximity of the Me-23, H-2ax, and Me-25 to the C-24 hydroxymethyl protons which show the axial configuration of the hydroxymethyl group at C-4.

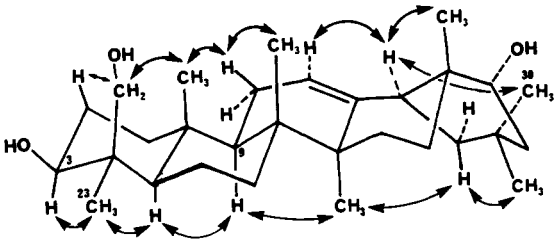


FIGURE 3. Transannular and interannular nOe interactions in the ROESY-45 spectrum of **2**.

The combination of H-3ax/Me-23 and 24-CH_2OH/Me-25 nOe's, which can also be observed in single dimension difference nOe spectra at lower fields, may provide a useful tool for routine assignment of C-4 stereochemistry in this type of molecule. In contrast, difference nOe spectra of hederagenin [**6**], which possesses the opposite configuration at C-4, exhibit H-3ax/23-CH_2OH and Me-24/Me-25 interactions (data not shown).

The data presented here demonstrate unambiguously that the olean-12-ene triterpenoid aglycone of several soyasaponins present in alfalfa seed is oxygenated on the C-24 carbon atom and is therefore soyasapogenol B; other saponins present from the same source contain aglycones with a C-23 oxygenated function. The biosynthetic origins of these complementary triterpenoids have yet to be fully elucidated, and the importance of oxygenation at C-23 or C-24 for anti-nutritional and plant protection properties has yet to be determined.

EXPERIMENTAL

PLANT MATERIAL AND ISOLATION.—Saponins were isolated from milled alfalfa and pea seeds as described previously (4). Each flour (100 g) was extracted with H_2O (300 ml) at room temperature using an Ultra Turrax blender. The supernatant was subjected to sequential elution through a reversed-phase silica (C_{18}) column with H_2O and MeOH. The MeOH eluate was evaporated under reduced pressure, and the residue (100 mg) was hydrolyzed with anhydrous methanolic HCl. The products were extracted into EtOAc, which was then removed under reduced pressure. The sapogenol mixture so obtained was chromatographed on a Si gel column with $CHCl_3$, and fractions containing soyasapogenol B were bulked and purified using preparative tlc to give crystalline soyasapogenol B [**2**]. Its identity was confirmed by comparison (nmr, ms) with authentic [**2**] isolated from soya flour (4).

GENERAL.—^{13}C DEPT (6) and APT (7) experiments were carried out on a Varian VXR-600 spectrometer. COSY and ROESY experiments were carried out on Varian VXR-300 and VXR-500 spectrometers, respectively. For the ROESY-45 experiment the spectral width was 3800 Hz in F_1 and F_2, the data set 2048 × 400 points, and the mixing time 200 msec. The spectrum was recorded in the absorption mode. Difference nOe experiments were carried out on a Bruker WM-360 spectrometer. All spectra were recorded in $CDCl_3$.

ACKNOWLEDGMENTS

We thank Drs. A. Farmer and J. Shoolery of Varian Instruments, Palo Alto, California for nmr spectra, Professor M. Jurzysta, IUNG, Pulawy, Poland, for a gift of hederagenin, and Dr. I. Colquhoun, IFR, Norwich, for preliminary data and helpful discussions.

LITERATURE CITED

1. K.R. Price, I.T. Johnson, and G.R. Fenwick, *CRC Crit. Rev. Food Sci. Nutr.*, **26**, 27 (1987).
2. A. Kadota and Y. Uchida, *Jpn. Kokao Tokkyo Koho* JP 6212792 (1986); *Chem. Abstr.*, **107**, 195156p (1987).
3. A.C. Dorsaz and K. Hostettmann, *Helv. Chim. Acta*, **69**, 2038 (1986).
4. K.R. Price, C.L. Curl, and G.R. Fenwick, *J. Sci. Food Agric.*, **37**, 1185 (1986).
5. I. Kitagawa, M. Yoshikawa, H.K. Wang, M. Saito, V. Tosirisuk, T. Fujiwara, and K. Tomita, *Chem. Pharm. Bull.*, **30**, 2294 (1982).
6. G. Massiot, C. Lavaud, D. Guillaume, and L. Le Men-Olivier, *J. Agric. Food Chem.*, **36**, 902 (1988).
7. K. Tori, S. Seo, A. Shimaoka, and Y. Tomita, *Tetrahedron Lett.*, 4227 (1974).
8. S. Seo, Y. Tomita, and K. Tori, *Tetrahedron Lett.*, 7 (1975).
9. K. Tori, Y. Yoshimura, S. Seo, K. Sakurawai, Y. Tomita, and H. Ishii, *Tetrahedron Lett.*, 4163 (1976).
10. H. Ishii, K. Tori, T. Tozyo, and Y. Yoshimura, *J. Chem. Soc., Perkin Trans 1*, 1928 (1981).
11. Y. Chen, T. Takeda, Y. Ogihara, and Y. Iitaka, *Chem. Pharm. Bull.*, **32**, 3378 (1984).
12. Y. Chen, T. Takeda, and Y. Ogihara, *Chem. Pharm. Bull.*, **33**, 1043 (1985).
13. D.T. Pegg, D.M. Doddrell, and M.R. Bendall, *J. Chem. Phys.*, **77**, 2745 (1982).
14. S.L. Patt and J.N. Shoolery, *J. Magn. Reson.*, **46**, 535 (1982).
15. A. Bax and G.A. Morris, *J. Magn. Reson.*, **42**, 501 (1981).
16. A. Bax, *J. Magn. Reson.*, **53**, 517 (1983).
17. B. Danieli, G.P. Forni, G. Palmisano, G. Rainoldi, and G.S. Ricca, *Chem. Ind.*, 748 (1974).
18. A. Bax and D.G. Davis, *J. Magn. Reson.*, **63**, 207 (1985).

Received 26 May 1989