Arch. Pharm. Res. **1**(1), 21~25(1978)

Carbon-13 NMR Spectra of Phytolaccagenin and its Glycosides

Won Sick Woo, Sam Sik Kang, Kazuo Yamasaki* and Osamu Tanaka*

*Natural Products Research Institute, Seoul National University, Seoul 110, Korea and Institute of Pharmaceutical Sciences, Hiroshima University, Japan**

(Received 10 November 1978)

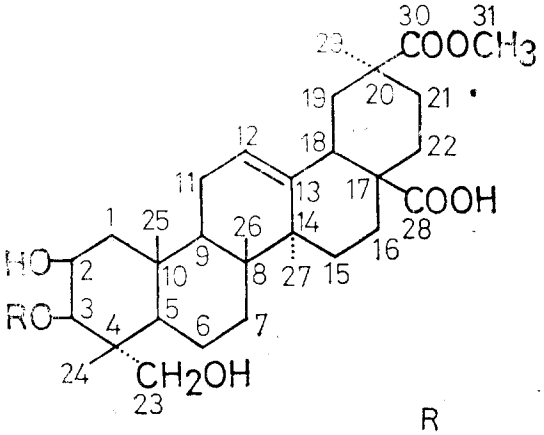
Abstract □ In connection with structure studies on triterpenoid glycosides from *Phytolacca* plants, full assignments of ^{13}C-NMR signals of phytolaccagenin and its glycosides, phytolaccoside B and E, have been presented.

Keyphrases □ —Phytolaccoside E and B—triterpenoidal glycosides—phytolaccagenin—^{13}C-NMR spectral analysis.

The roots of *Phytolacca* plants have been used as a folk medicine in treating edema and rheumatism, being found rich in saponins named phytolaccosides,[1] with strong anti-inflammatory activity.[2]

Five saponins, phytolaccosides A, B, D, E and G were isolated from *Phytolacca americana* and identified as 3-O-β-D-xylopyranosyl-esculentic acid 30-methylester, 3-O-β-D-xylopyranosyl-jaligonic acid 30-methylester, 3-O-[β-D-glucopyranosyl (1→4)-β-D-xylopyranosyl]-esculentic acid 30-methylester, 3-O-[β-D-glucopyranosyl (1→4)-β-D-xylopyranosyl]-jaligonic acid 30-methylester and 3-O-D-xylopyranosyl-jaligonic acid, respectively.[3,4] *Phytolacca* saponins E and G reported by Suga *et al.*[5] are identical with our phytolaccosides E and B. From the viewpoint of chemotaxonomy, it is worth to note that the highly oxygenated 28,30-dicarboxyl-pentacyclic triterpenoids in these saponins as aglycone have been found only in the genera *Phytolacca*,[6] *Mollugo*[7] and *Serjanica*.[8]

Our current interest in the biogenesis of these saponins prompted us to undertake ^{13}C-NMR spectral analysis which permits the use of ^{13}C-enriched precursors in biosynthetic studies. We present here full signal



	R
Phytolaccagenin	H
Phytolaccoside B	β-Xyl
Phytolaccoside E	β-Glu(1→4)-β-Xyl

Table I. ^{13}C Chemical Shift (δ_C) in C_5D_5N from internal TMS

Carbon No:	Phytolaccagenin (1)	Phytolaccoside-B (2)	$\Delta\delta=\delta_2-\delta_1$	Phytolaccoside-E (3)	$\Delta\delta=\delta_3-\delta_2$
1	44.8	44.1	−0.7	44.1	
2	71.5	71.0	−0.5	70.8	
3	73.0	82.9	+9.9	82.9	
4	42.4	42.8	+0.4	42.8	
5	48.1	47.7		47.6	
6	18.2	18.0		17.9	
7	33.0	33.0		32.9	
8	39.8	39.8		39.8	
9	48.5	48.5		48.5	
10	37.2	37.0		36.9	
11	23.9	23.9		23.8	
12	123.3	123.5		123.2	
13	144.4	144.3		144.4	
14	42.2	42.2		42.2	
15	28.4	28.4		28.4	
16	23.9	23.9		23.8	
17	46.1	46.1		46.1	
18	43.3	43.4		43.3	
19	42.7	42.8		42.7	
20	44.1	44.1		44.1	
21	30.8	30.8		30.8	
22	34.5	34.5		34.4	
23	67.7	65.3	−2.4	65.1	
24	14.5	14.9	+0.4	14.9	
25	17.4[a]	17.4		17.4[a]	
26	17.2[a]	17.4		17.2[a]	
27	26.2	26.2		26.2	
28	179.7	179.7		179.8	
29	28.4	28.4		28.4	
30	177.1	177.1		177.1	
31 (OMe)	51.6	51.6		51.6	
Xyl-1	106.1[c]	106.7		106.2	−0.5
2	74.6	75.3		74.9	−0.4
3	78.1	78.4		76.3	−2.1
4	70.9	71.0		77.5	+6.5
5	66.9	67.2		64.6	−2.6
Glc-1	105.7[d]			103.4	
2	74.9			74.2	
3	78.3			78.7[b]	
4	71.6			71.6	
5	78.3			78.0[b]	
6	62.7			62.6	

a,b Values may be reversed.

c,d Values for methyl-β-D-xylopyranoside[18] and methyl-β-D-glucopyranoside,[17] respectively.

assignments for proton-noise decoupled natural-abundance ^{13}C FT NMR spectra of two major saponins, phytolaccoside B and E, and their aglycone, phytolaccagenin.

The signal assignments were generally carried out by means of single-frequency off-resonance decoupling techniques,[9] proton selective decoupled experiments,[10] relaxation time measurements,[11,12] and chemical shift comparisons from compound to compound using the previously reported data of oleanene type triterpenoids,[10,13-16] β-D- glucopyranoside[17] and β-D-xylopyranoside,[18] and the substitution reguralities, such as hydroxyl substitution shifts,[10,14,15,19] carbomethoxyl substitution shifts,[20] and glycosidation shifts.[18,21-23] The δ values determined are listed in Table I.

Carboxyl carbon signals were readily characterized from their chemical shifts: of the two signals, the higher field one was assigned to C-30, since free carboxylic acid carbon resonated further downfield compared with ester carbon.[20] Signals due to the olefinic carbons were also identified on the basis of their chemical shifts and multiplicities. The six high field singlets for quarternary carbons which were easily observed in the off-resonance decoupling spectra were readily be identified by comparison with hederagenin methylester[10] as a reference compound: two resonances at lower field (42.4 and 44.1 ppm in the spectrum of phytolaccagenin) were assigned to C-4 and C-20, respectively, since C-4 resonance should undergo slight change (+0.4ppm) by glycosidation at C-3. Five higher field resonances corresponding to angular methyl carbons

were also resolved, except two resonances (at 17.4 and 17.2ppm), which remained unassigned and might be due to C-25 and C-26. The lowest of the methylene signals should correspond to C-1, on the basis of β-substitution effect of C-2 hydroxylation.

Two methine carbon resonances at 48.1 and 48.5 ppm in the spectrum of phytolaccagenin could be assigned to C-5 and C-9, respectively, since the signal for C-9 did not change on going from the aglycone to glycosides.

The identification of the other methine and methylene carbon signals was permitted by the comparison of reported data for oleanene type triterpenes.[10, 13-16]

Identification of the signals due to C-2 and C-3 carbinyl carbons was secured by proton slective decoupling technique.

In the ^{1}H NMR spectrum of phytolaccagenin (in C_5D_5N), signals due to H-2 and H-3 appeared at δ 4.57 (1H, m, $W_{1/2}$=8Hz) and 4.28 (1H, d, J=4Hz), respectively. The doublets due to C-2 and C-3 were clearly seen to collapse into singlets by the irradication of H-2 and H-3, respectively. Interestingly, C-3 signal of phytolaccagenin was not displaced downfield by introducing an axial OH group at C-2 but appeared at unusually high field. This abnormal shift could not be immediately explained.

In comparison of ^{13}C NMR spectrum of phytolaccoside B with that of phytolaccagenin, a set of newly appeared signals in the sugar carbon region of the spectrum of phytolaccoside B was completely identified by comparison with reported data for methyl-β-D-xylopyranoside.[18] As expected, on going from phytolaccagenin to phytolaccoside B, the signal due to C-3 was displaced downfield by+9.9 ppm and the one due to C-23 was shielded by−2.4 ppm, while other resonances appeared almost unshifted (signals due to the C-2 and C-4 were shifted by −0.5 and +0.4 ppm respectively). The glycosidation shift values were in good agreement with the reported data.[18, 21-23]

In comparison of the spectrum of phytolaccoside E with that of B, all of the signals due to the sapogenin moiety of both compounds appeared at almost the same positions.

With regard to the sugar carbon region, the six carbon signals of phytolaccoside E having relatively longer relaxation time (T_1) by PRFT could be assigned as a terminal β-D-glucopyranoside and characterization of each of these resonances was established by comparison with the reported data for methyl-β-D-glucopyranoside.[17] The other carbon resonances in this region of the spectrum of phytolaccoside E, which were definitely distinguished from those of the aforementioned β-D-glucopyranoside unit because of their shorter T_1 by PRFT, could be assigned to the 4′-substituted β-D-xylopyranoside; on going from phytolaccoside B to E, the signal of C-4′ of D-xylose moiety was deshielded by +6.5ppm, while signals due to the C-3′ and C-5′ were shielded by −2.1 and −2.6ppm, respectively.

EXPERIMENTAL

^{13}C NMR spectra were recorded on a JEOL PFT 100 spectrometer equipped with

JEC-6 computer at 25.149 MHz in C_5D_5N with TMS as an internal reference in 10mm spinning tubes at 25°; concentration was 0.15–0.2 mmol/ml. FT measurement conditions were as follows: spectral width, 5000 Hz; pulse flipping angle, 90°; acquisition time, 0.4 sec; number of data points, 8192.

LITERATURE CITED

1) Woo, W. S., Shin, K. H., and Kang, S. S., Constituents of *Phytolacca* species. (1). Antiinflammatory saponins. *Kor. J. Pharmacogn.* **7,** 47(1976).
2) Woo, W. S., and Shin, K. H., Antiinflammatory action of *Phytolacca* saponin. *J. Pharm. Soc. Korea* **20,** 149(1976).
3) Woo, W. S., and Kang, S. S., The structure of phytolaccoside G. *J. Pharm. Soc. Korea* **21,** 159(1977).
4) Woo, W. S., Kang, S. S., Wagner, H. Seligmann, O., and Chari, V. M., Triterpenoid saponins from the roots of *Phytolacca americana*. *Planta Med.* **34,** 87(1978).
5) Suga, Y., Maruyama, Y., Kawanishi, S., and Shoji, J., Studies on the structures of phytolaccasaponin B, E and G from the roots of *Phytolacca americana* L. *Chem. Pharm. Bull.* **26,** 520(1978).
6) Woo, W. S., and Kang, S. S., The occurrence and chemistry of Phytolacca triterpenoids. *J. Pharm. Soc. Korea* **19,** 189(1975).
7) Chakrabarti, P., Makherjee, D. K., Chatterjee, R., and Barua, A. K., Isolation of spergulagenic acid - A new sapogenin from *Mollugo spergula*. *Indian J. Chem.* **3,** 283(1965).
8) Savoir, R., Tursch, B., and Kaisin, M., Triterpenes. XIII. Serjanic acid, a new triterpene from the Sapindaceae. *Tetrahedron Lett.* 2129(1967).
9) Reich, H. J., Jautelat, M., Messe, M. T., Weigert, F. G., and Roberts, J. D., Nuclear magnetic resonance spectroscopy. Carbon-13 spectra of steroids. *J. Am. Chem. Soc.* **91,** 7445(1976).
10) Tori, K., Seo, S. Shimaoka, A., and Tomita, Y., Carbon-13 NMR spectra of olean-12-enes. Full signal assignments including quaternary carbon signals assigned by use of indirect ^{13}C, ^{1}H spin couplings. *Tetrahedron Lett.* 4227(1974).
11) Allerhand, A., and Doddrell, D., Strategies in the application of partially relaxed Fourier transform nuclear magnetic resonance spectroscopy in assignments of carbon-13 resonances of complex molecules. *J. Am. Chem. Soc.* **93,** 2777(1971).
12) Yahara, S. Kasai, R., and Tanaka, O., New dammarane type saponins of leaves of *Panax japonicus* C. A. Meyer (1). Chikusetsusaponins-L_5, -L_{9a} and -L_{10}. *Chem. Pharm. Bull.* **25,** 2041(1977).
13) Doddrell, D. M., Khong, P. W., and Lewis, K. G., The stereochemical dependence of ^{13}C chemical shifts in olean-12-enes and urs-12-enes as an aid to structural assignments. *Tetrahedron Lett.* 2381 (1974).
14) Seo, S., Tomita, Y., and Tori, K., Carbon-13 NMR spectra of urs-12-enes and application to structural assignments of components of *Isodon japonicus* Hara tissue cultures. *Tetrahedron Lett.* 7(1975).
15) Tori, K., Yoshimura, Y., Seo, S., Sakurawi, K., Tomita, Y., and Ishii, H., Carbon-13 NMR spectra of saikogenins. Stereochemical dependence in hydroxylation effects upon carbon-13 chemical shifts of oleanene-type triterpenoids. *Tetrahedron Lett.* 4163(1976).
16) Ishii, H., Tori, K., Tozyo, T., and Yoshimura, Y., Structures of prosapogenins from roots of *Platycodon grandiflorum* determined by carbon-13 and hydrogen-1 nuclear magnetic resonance spectroscopy. *Chem. Pharm. Bull.* **26,** 671(1978).
17) Yamasaki, K., Kohda, H., Kobayashi, T., Kasai R., and Tanaka, O., Structure of Stevia diterpene-glucosides; Application of ^{13}C-NMR. *Tetrahedron Lett.* 1005(1976).
18) Seo, S., Tomita, Y., Tori, K., and Yoshimura, Y., Determination of the absolute configuration of a secondary hydroxy group in a chiral secondary alcohol using glycosidation shifts in carbon-13

nuclear magnetic resonance spectroscopy. *J. Am. Chem. Soc.* **100**, 3331(1978).

19) Eggert, H. Van Antwerp, C. L., Bhacca, N. S., and Djerassi, C., Carbon-13 nuclear magnetic resonance spectra of hydroxy steroids. *J. Org. Chem.* **41**, 71(1976).

20) Grutzner, J. B., Jautelat, M., Dence, J. B., Smith, R. A., and Roberts, J. D., Nuclear magnetic resonance spectroscopy. Carbon-13 chemical shifts in norbornyl derivatives. *J. Am. Chem. Soc.* **92**, 7107(1970).

21) Tori, K., Seo, S., Yoshimura, Y., Nakamura, M., Tomita, Y., and Ishii, H., Carbon-13 NMR spectra of saikosaponins A, C, D and F. *Tetrahedron Lett.* 4167(1976).

22) Kasai, R., Suzuo, M., Asakawa, J., and Tanaka, O., Carbon-13 chemical shifts isoprenoid-β-D-glucopyranosides and -β-D-manopyranosides. Stereochemical influences of aglycone alcohols. *Tetrahedron Lett.* 175(1977).

23) Tori, K., Seo, S., Yoshimura, Y., Arita, H. and Tomita, Y., Glycosidation shifts in Carbon-13 NMR spectroscopy: Carbon-13 signal shifts from aglycone and glucose to glucosine. *Tetrahedron Lett.* 179(1977).