^{13}C NUCLEAR MAGNETIC RESONANCE SPECTRA OF CORILAGIN AND GERANIIN[1]

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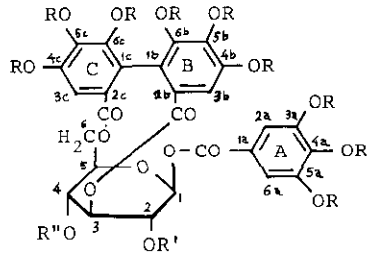
Abstract ——— The ^{13}C nmr spectra of corilagin and its derivatives have been recorded and the signals of all carbons assigned. The analysis has been extended to geraniin.

^{13}C Nuclear magnetic resonance (^{13}C nmr) spectroscopy has been found in our research[2] as a much more informative tool than ^{1}H nmr on the structural elucidation of ellagitannins, which have many polyphenolic carbons in spite of a small number of C-H bondings in a molecule. The present paper deals with the detail of ^{13}C nmr assignments of corilagin (**1**)[3] and extention of the analysis to geraniin (**2**)[2].

The ^{13}C chemical shifts of corilagin and geraniin are summarized in Table 1. Among the signals due to the aromatic carbons in corilagin (**1**), the carbon signals of ring A were assigned by comparison with the signals of methyl gallate[4], and the relative peak heights. Two carbons which resonate at higher field than C-2b and C-2c were assigned to C-1b and C-1c based on the substituent chemical shift theory[5]. These assignments were confirmed by analysis of the fully proton-coupled spectrum, in which each peak of C-1b and C-1c was exhibited as a doublet (J 7 Hz) as the result of a CCCH coupling, while the peaks due to C-2b and C-2c were shown as singlets. The fully coupled spectrum also distinguished the resonance of C-4a (t, J 6 Hz) from those due to C-5b and C-5c (d, J 8 Hz). However, pairs of carbons of equivalent position in ring B and C were not mutually distinguishable.

The assignments of the signals of glucose moiety of **1** is troubled by the perturbed boat conformation[6] of the glucopyranose ring in **1**. The complete analysis of the glucose carbons in Table 2 was made by application of the



1 R=R'=R"=H
3 R=Me, R'=R"=H
4 R=R'=Me, R"=H
5 R=R"=Me, R'=H
6 R=R'=R"=Me
7 R=Me, R'=CONHMe, R"=H
8 R=Me, R'=H, R"=CONHMe
9 R=R'=Me, R"=CONHMe

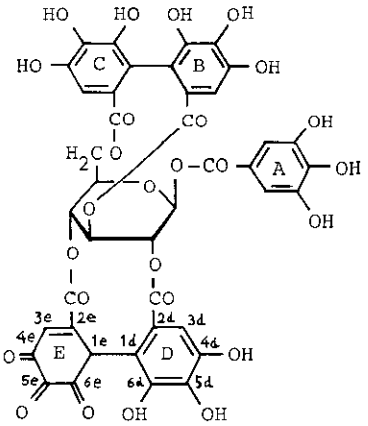


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Table 1. ^{13}C Chemical shifts of corilagin (**1**) and geraniin (**2**) in acetone-d_6

	carbon*	**1**	**2**		carbon	**1**	**2**
ring A	1a	121.6	120.2		5d		139.0
	2a (6a)	111.7	110.9		6d		143.4
	3a (5a)	146.3	146.0				
	4a	140.2	139.9	ring E	1e		46.2
					2e		154.5
ring B	1b	117.3	115.8		3e		128.6
and	1c	116.7	117.2		4e		191.8
ring C	2b	126.5	125.6		5e		96.3
	2c		124.6		6e		92.5
	3b	111.1	110.6				
	3c	109.1	108.0	glucose	1	95.0	90.8
	4b	145.8	145.8		2	69.2	72.7
	4c		145.5		3	70.7	70.7
	6b		145.1		4	62.5	65.9
	6c		145.0		5	76.0	63.3
	5b	138.1	137.9		6	64.8	63.8
	5c	137.6	136.6				
				ester		169.8	168.4
ring D	1d		115.3			168.4	166.1
	2d		119.4			166.3	165.6
	3d		113.5				165.4
	4d		144.6				164.8

*The numbering in rings B~E has been based on that in ref. 1.

deuterium-induced differential isotope shift (DIS)[7] study, and the substitution-induced shift. The anomeric carbon and C-6 signals were readily assigned on the basis of the chemical shifts and the multiplicities in the off-resonance spectrum. The signals due to C-2 and C-4, both of which carry free hydroxyl group, are unambiguously differentiated from the others by the DIS measurement of **1**, upon which each one of the signals at 69.2 and 62.5 ppm appear as double signals with significant DIS value of 0.22 ppm each. Differentiation of C-2 and C-4, and assignments of C-3 and C-5 were based on the substitution shifts observed for several derivatives of nona-O-methylcorilagin (**3**).

Upon treatment of **3** with diazomethane in the presence of a small amount of stannous chloride[8], two isomeric deca-O-methylcorilagin (**4**) and (**5**), between which the former was the major product, were obtained. These structures were established by methanolysis which yielded 2-O-methyl-D-glucopyranose and 4-O-methyl D-glucopyranose. Permethylated derivative (**6**) was prepared by methylation of **3** or **4** with diazomethane-boron trifluoride etherate. When diazomethane prepared from nitrosomethylurea was employed without drying for methylation of **3**, two isomeric mono-O-methylcarbamoyl derivatives, (**7**) and (**8**) were produced by the reaction with methyl isocyanate which was present in the reagent[9]. However, di-O-methylcarbamoyl derivative was not detected under this condition, and none of the mono-O-methylcarbamoyl derivatives was produced when dry diazomethane solution was used for methylation. Methylation of **8** gave methyl derivative (**9**), which was identified with the specimen obtained by the treatment of **4** with methyl isocyanate. Thus, the structures of **7** and **8** were characterized as 2-O-methylcarbamoyl-nona-O-methylcorilagin and 4-O-methylcarbamoyl-nona-O-methylcorilagin, respectively.

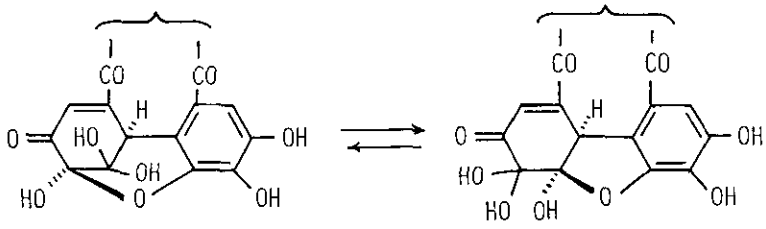
Table 2. ^{13}C Chemical shifts of glucose carbons of **1** and its derivatives

carbon	1*	3	4	6	7	8
1	95.0	96.4	93.5	93.0	92.5	95.6
2	69.2	67.7	76.4	76.5	69.1	67.7
3	70.7	69.3	67.4	66.4	68.6	68.6
4	62.5	62.6	61.3	68.8	61.9	64.0
5	76.0	74.9	76.0	72.3	75.0	72.6
6	64.8	64.4	64.4	63.7	64.0	64.2

*Measured in acetone-d_6, the others were measured in $CDCl_3$.

Based on the methylation shift[10], the signals of **3** at 67.7 and 69.3 ppm were assigned to C-2 and C-3, respectively, since on methylation of **3** to **4**, the former peak shifted downfield by 8.5 ppm, while the latter peak was shielded by 1.9 ppm. Similar methylation shifts were observed upon the formation of **6** from **4**, and allowed the assignments of the signals of 3 at 62.6 and 74.9 ppm to C-4 and C-5. These assignments were further confirmed by the reasonable acylation shifts observed upon the formation of **7** or **8** from **3** as shown in Table 2.

The ^{13}C nmr analysis was extended to geraniin which is the main tannin of Geranium thunbergii[2]. Structure of geraniin is fundamentally shown by **2**. The ^{13}C chemical shifts of **2** in Table 1 were measured in a short time after dissolution of crystalline geraniin in acetone-d_6. The carbon resonances due to the aromatic carbons of corilagin moiety of 2 can be readily characterized by comparison with those of **1**, and the remaining signals are regarded as those of ring D and E. These tentative assignments were verified by comparison of the spectra before and after the equilibration in acetone-d_6-D_2O, since the chemical shift changes on the equilibration (**2a** $\rightleftarrows$ **2b**)[2] were most distinct in the carbon resonances of ring D and E. The resonances at 191.8, 154.5 and 128.6 ppm were unambiguously assigned to those of α,β-unsaturated ketone system on the basis of the chemical shifts and multiplicities in the off-resonance spectrum. No other ketone carbonyl signal except that at 191.8 ppm was observed in the spectrum of **2**, indicating that two of the three ketones are hydrated to form gem-diol and hemi-acetal[2], whose resonances can be associated with the signals at 92.5 and 96.3 ppm. The detail of the analysis of partial structure **2a** $\rightleftarrows$ **2b** will be described in a subsequent paper.



2a **2b**

EXPERIMENTAL

Mps were taken on a Yanagimoto micro-melting point apparatus and are uncorrected. Uv spectra were measured on a Hitachi Spectrophotometer 200-10. ^{1}H nmr spectra were recorded on a Hitachi R-22 or R-22 FTS (90 MHz) spectrometer, and chemical shifts are given in ppm relative to TMS as an internal standard. Mass spectra (ms) were obtained with a Shimadzu-LKB 9000 GC-MS equipped with 2 m x 3 mm i.d. glass column containing 2.5% OV-17 on Chromosorb W and direct inlet system. Gas chromatographic analysis was carried out on a Hitachi Gas Chromatograph 163 equipped with 2 m x 3 mm i.d. glass column packed with 2.5% OV-1 on Chromosorb W. Specific rotation at the D line was determined using a JASCO DIP-4 Digital Polarimeter. Kieselgel PF_{254} (Merck) was used for analytical and preparative TLC. Solvent system used for TLC was benzene-acetone (4:1) unless otherwise stated, and compounds were detected by viewing under uv light. The organic solutions were dried over $MgSO_4$ and evaporated in rotary evaporator under 40°.

^{13}C Nmr spectral measurement ——— ^{13}C Nmr spectra were measured at 22.6 MHz at 34° with a Hitachi R-22 FTS Spectrometer at concentrations of approximately 0.3-0.5 M in a microtube (0.3 ml). On average, pulse angle 40°, pulse interval 3 sec, spectral width 5400 Hz, and 16 K data points were employed. DIS spectra were obtained with a dual sample tube consisting of 5 mm tube (0.4 M in acetone-d_6-H_2O, 3:2) and 8 mm tube (0.4 M in acetone-d_6-D_2O, 3:2).

Methylation of 3 in the presence of stannous chloride ——— Nona-O-methylcorilagin (3) (100 mg) in MeOH (10 ml) containing $SnCl_2$ (2 mg) was treated with excess diazomethane (in ether dried over KOH pellets) at room temperature for 4 h. After removal of the solvent, the residue was fractionated by prep. TLC (solvent: benzene-acetone, 85:15, double development) to give two isomeric deca-O-methyl ethers, (4) (53.1 mg) and (5) (21.4 mg), along with methyl tri-O-methylgallate (6 mg), dimethyl hexamethoxydiphenoate (2 mg) and the starting material (5.6 mg). Recrystallization of 4 from EtOH-ether afforded colorless fine needles, mp 136-138°, $[\alpha]_D^{20}$ -121.4° (c 0.58, $CHCl_3$). uv λ_{max}^{MeOH} nm (log ε): 216 (4.69), 250 (4.24), 290 (sh)(3.89). ^{1}H nmr ($CDCl_3$) δ: 3.11-3.93 (10 x OMe), 6.55 (1H, br.s, gluc.-H_1), 6.47, 6.74 (1H each, s), 7.20 (2H, s). ms (m/e): 774 (M^+), 562 (M^+-212). Anal. Calcd. for $C_{37}H_{42}O_{18}$: C, 57.31; H, 5.42. Found: C, 57.54; H, 5.75. Crystallization of 5 from acetone gave colorless needles, mp 231-233°, $[\alpha]_D^{20}$ -142° (c 0.86, $CHCl_3$). uv λ_{max}^{MeOH} nm (log ε): 216 (4.69), 250 (4.28), 290 (sh)(3.89).

^{1}H nmr ($CDCl_3$) δ: 3.24-3.98 (10 x OMe), 6.53 (1H, br.s, gluc.-H_1), 6.60, 6.76 (1H each, s), 7.22 (2H, s). ms (m/e): 774 (M^+), 562 (M^+-212). Anal. Calcd. for $C_{37}H_{42}O_{18}$: C, 57.31; H, 5.42. Found: C, 57.05; H, 5.52.

Methanolysis of **4** and **5** ——— To a solution of **4** (2 mg) in dry MeOH (0.3 ml), 1 % NaOMe solution (0.1 ml) was added, and the reaction mixture was kept overnight at room temperature. After neutralization with IR-120 resin followed by filtration, the solvent was evaporated and the residue was partitioned between $CHCl_3$ and H_2O. Water layer was evaporated to yield a syrupy residue which was submitted to GC and GC-MS analysis after trimethylsilylation, and was identified with authentic 2-O-methyl-D-glucopyranose by the two peaks of anomeric mixtures.

The sugar component obtained from **5** by methanolysis as described above was identified with authentic 4-O-methyl-D-glucopyranose.

Undeca-O-methylcorilagin (**6**) ——— Nona-O-methylcorilagin (**3**) (80 mg) was dissolved in dry CH_2Cl_2 (2 ml). The solution was kept at -5°, and boron trifluoride etherate (8 μl) was added. An excess of ethereal diazomethane was then added until faint yellow color persisted in the solution. After 2 h at -5°, the solid (polymethylene) was filtered off, and the filtrate was evaporated. The residue was purified by prep. TLC to give the starting material (10 mg), and **6** (44 mg) as colorless fine needles, mp 199-200°, $[\alpha]_D^{20}$ -145° (c 1.08, $CHCl_3$). uv λ_{max}^{MeOH} nm (log ε): 218 (4.78), 254 (4.43). ^{1}H nmr ($CDCl_3$) δ: 3.31-3.98 (11 x OMe), 6.56 (1H, br.s, gluc.-H_1), 6.58, 6.80 (1H each, s), 7.23 (2H, s). ms (m/e): 788 (M^+), 576 (M^+-212). Anal. Calcd. for $C_{38}H_{44}O_{18}$: C, 57.86; H, 5.62. Found: C, 57.62; H, 5.39.

Preparation of **7** and **8** ——— To a solution of **3** (120 mg) in MeOH (1 ml), ethereal diazomethane which was prepared from nitrosomethylurea (not dried) was added. After 10 h at room temperature, the solvent was removed and the residue was treated again with ethereal diazomethane for 20 h. Evaporation and purification by prep. TLC (double development) gave two isomeric methylcarbamates, (**7**) (42 mg) and (**8**) (22.5 mg). Mehtylcarbamate (**7**): mp 249-251° from MeOH, $[\alpha]_D^{20}$ -129° (c 1, $CHCl_3$). uv λ_{max}^{MeOH} nm (log ε): 218 (4.68), 250 (sh)(4.30), 292 (3.90). ^{1}H nmr ($CDCl_3$) δ: 2.83 (3H, d, J 5 Hz, CONHMe), 3.43-3.96 (10 x OMe), 6.51 (1H, br.s, gluc.-H_1), 6.71, 6.78 (1H each, s), 7.22 (2H, s). ms (m/e): 605 (M^+-212), 548 (605-57), 530, 422, 212. Anal. Calcd. for $C_{38}H_{43}O_{19}N$: C, 55.81; H, 5.30; N, 1.71. Found: C, 55.39; H, 5.17; N, 1.51. Methylcarbamate (**8**): mp 221-222°, $[\alpha]_D^{20}$

-122° (c 0.98, $CHCl_3$). uv λ_{max}^{MeOH} nm (log ε): 219 (4.72), 250 (sh)(4.36), 295 (sh) (3.95). ^1H nmr ($CDCl_3$) δ: 2.81 (3H, d, J 5 Hz, CONHMe), 3.31-3.98 (9 x OMe), 6.52 (1H, br.s, gluc.-H_1), 6.61, 6.90 (1H each, s), 7.24 (2H, s). ms (m/e): 605 (M^+-212), 548 (605-57), 530, 422, 212. Anal. Calcd. for $C_{38}H_{43}O_{19}N \cdot H_2O$: C, 54.61; H, 5.39; N, 1.68. Found: C, 54.16; H, 5.19; N, 2.04.

Methylation of **8** ——— To a solution of **8** (20 mg) in dry CH_2Cl_2 (2 ml) was added ethereal diazomethane (dried over KOH pellets). Boron trifluoride etherate (20 μl) was then added at -5°. After 1 h, white solid (polymethylene) was filtered off, and the filtrate was evaporated. The crystalline residue was purified by prep. TLC to afford the starting material (2 mg), and methyl ether (**9**) (10 mg) as colorless needles, mp 237-239°, $[\alpha]_D^{20}$ -129° (c 0.88, $CHCl_3$). uv λ_{max}^{MeOH} nm (log ε): 215 (4.72), 250 (sh)(4.10), 294 (sh)(4.30). ^1H nmr ($CDCl_3$) δ: 2.82 (3H, d, J 5.5 Hz, CONHMe), 3.31-4.00 (10 x OMe), 6.53 (1H, br.s, gluc.-H_1), 6.58, 6.90 (1H each, s), 7.25 (2H, s). ms (m/e): 831 (M^+), 774 (M^+-57), 619 (M^+-212).

Preparation of **9** from **4** ——— A solution of **4** (20 mg) and methyl isocyanate (40 μl) in CH_2Cl_2 (2 ml) containing pyridine (0.1 ml) was kept at 40° for a week. After removal of the solvent, the residue was purified by prep. TLC to give the starting material (11 mg), and **9** (7.3 mg) which formed colorless needles, mp 236-237°, and was identified with the compound obtained above by mixed mp, ms, $[\alpha]_D$ and ^1H nmr.

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