

^{13}C NUCLEAR MAGNETIC RESONANCE SPECTRA OF CORILAGIN AND GERANIIN¹

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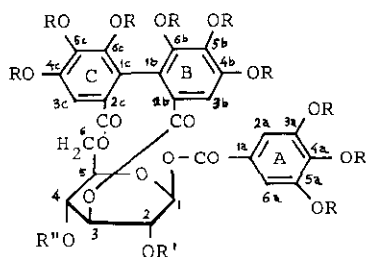
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Abstract — The ^{13}C nmr spectra of corilagin and its derivatives have been recorded and the signals of all carbons assigned. The analysis has been extended to geraniin.

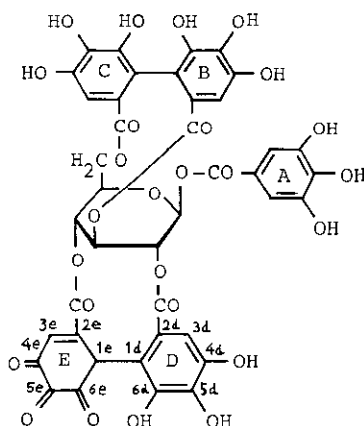
^{13}C Nuclear magnetic resonance (^{13}C nmr) spectroscopy has been found in our research² as a much more informative tool than ^1H nmr on the structural elucidation of ellagitannins, which have many polyphenolic carbons in spite of a small number of C-H bondings in a molecule. The present paper deals with the detail of ^{13}C nmr assignments of corilagin (1)³ and extension 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 gallate⁴, 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 theory⁵. 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 conformation⁶ 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



2

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	ring E	1e		46.2	
	4a	140.2	139.9		2e		154.5	
ring B	1b	117.3	115.8		3e		128.6	
	and 1c				116.7	117.2	4e	
ring C	2b	126.5	125.6	5e				96.3
	2c			111.1	110.6	6e		92.5
ring D	3b	109.1	108.0			glucose	1	95.0
	3c			145.8	145.8		2	69.2
	4b	145.5	145.1				3	70.7
	4c			145.0	145.1		4	62.5
	6b	138.1	137.9				5	76.0
	6c			137.6	136.6		6	64.8
	5b						ester	
	5c				168.4			166.1
ring E	1d			166.3	165.6			
	2d				165.4			
	3d				164.8			
	4d							

* 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 chloride⁸, 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 reagent⁹. 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.

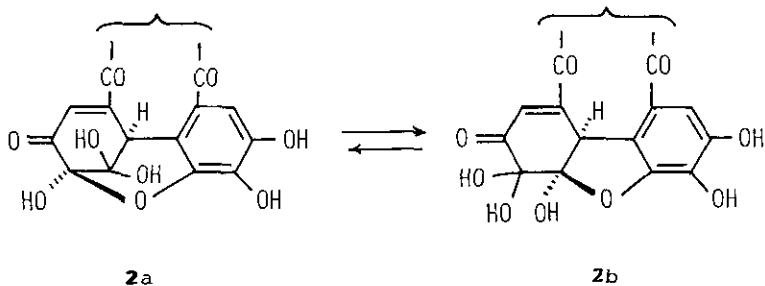
Table 2. ¹³C Chemical shifts of glucose carbons of **1** and its derivatives

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-d₆, the others were measured in CDCl₃.

Based on the methylation shift¹⁰, 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 ¹³C nmr analysis was extended to geraniin which is the main tannin of Geranium thunbergii². Structure of geraniin is fundamentally shown by **2**. The ¹³C chemical shifts of **2** in Table 1 were measured in a short time after dissolution of crystalline geraniin in acetone-d₆. 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-d₆-D₂O, since the chemical shift changes on the equilibration (**2a** ⇌ **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 hemiacetal², whose resonances can be associated with the signals at 92.5 and 96.3 ppm. The detail of the analysis of partial structure **2a** ⇌ **2b** will be described in a subsequent paper.



EXPERIMENTAL

Mps were taken on a Yanagimoto micro-melting point apparatus and are uncorrected. Uv spectra were measured on a Hitachi Spectrophotometer 200-10. ^1H 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 PF₂₅₄ (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 hexamethoxydiphenate (2 mg) and the starting material (5.6 mg). Recrystallization of 4 from EtOH-ether afforded colorless fine needles, mp 136-138°, $[\alpha]_{\text{D}}^{20}$ -121.4° (c 0.58, CHCl_3). uv $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 216 (4.69), 250 (4.24), 290 (sh) (3.89). ^1H 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 $\text{C}_{37}\text{H}_{42}\text{O}_{18}$: C, 57.31; H, 5.42. Found: C, 57.54; H, 5.75. Crystallization of 5 from acetone gave colorless needles, mp 231-233°, $[\alpha]_{\text{D}}^{20}$ -142° (c 0.86, CHCl_3). uv $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 216 (4.69), 250 (4.28), 290 (sh) (3.89).

^1H 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 $\text{C}_{37}\text{H}_{42}\text{O}_{18}$: 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 $199-200^\circ$, $[\alpha]_D^{20} -145^\circ$ (c 1.08, CHCl_3). uv $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 218 (4.78), 254 (4.43). ^1H 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 $\text{C}_{38}\text{H}_{44}\text{O}_{18}$: 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). Methylcarbamate (**7**): mp $249-251^\circ$ from MeOH, $[\alpha]_D^{20} -129^\circ$ (c 1, CHCl_3). uv $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 218 (4.68), 250 (sh) (4.30), 292 (3.90). ^1H 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 $\text{C}_{38}\text{H}_{43}\text{O}_{19}\text{N}$: C, 55.81; H, 5.30; N, 1.71. Found: C, 55.39; H, 5.17; N, 1.51. Methylcarbamate (**8**): mp $221-222^\circ$, $[\alpha]_D^{20}$

-122° (c 0.98, CHCl₃). uv $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 219 (4.72), 250 (sh) (4.36), 295 (sh) (3.95). ¹H nmr (CDCl₃) δ : 2.81 (3H, d, J 5 Hz, CONHMe), 3.31-3.98 (9 x OMe), 6.52 (1H, br.s, gluc.-H₁), 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 C₃₈H₄₃O₁₉N·H₂O: 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 CH₂Cl₂ (2 ml) was added ethereal diazomethane (dried over KOH pellets). Boron trifluoride etherate (20 μ l) 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°, [α]_D²⁰ -129° (c 0.88, CHCl₃). uv $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 215 (4.72), 250 (sh) (4.10), 294 (sh) (4.30). ¹H nmr (CDCl₃) δ : 2.82 (3H, d, J 5.5 Hz, CONHMe), 3.31-4.00 (10 x OMe), 6.53 (1H, br.s, gluc.-H₁), 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 μ l) in CH₂Cl₂ (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, [α]_D and ¹H nmr.

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