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Isolation of an Insect-Antifeedant, Phloretin 4′-*O*-β-D-Glucopyranoside, by Rotation Locular Counter-Current Chromatography and Determination of Its Preferred Conformation in Solution by Nuclear Magnetic Resonance Analysis

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A bitter insect-antifeedant dihydrochalcone, phloretin 4′-*O*-β-D-glucopyranoside, was isolated from fresh apple leaves by using rotation locular counter-current chromatography (RLCC), and the preferred conformation in solution was determined by means of proton (^{1}H-) and carbon-13 (^{13}C-) nuclear magnetic resonance (NMR) experiments.

Keywords——insect-antifeedant; phloretin 4′-*O*-β-D-glucopyranoside; rotation locular counter-current chromatography; conformation solution; NMR experiment

During our continuing investigation of naturally occurring pest control agents,[1] we found that the methanol extract of fresh leaves of the apple *Malus pumila* var. *dulcissima* MILL. (Rosaceae) showed antifeedant activity against a pest aphid species, the greenbug *Schizaphis graminum* RONDANI, based on an artificial diet feeding assay.[2] Although the chemical constituents in this plant have been studied,[3-5] it was not known which of the constituents was responsible for this observed biological activity. Separation of the crude extract into fractions soluble in *n*-hexane, ether, ethyl acetate, and water, followed by bioassay, indicated that the active component was in the ethyl acetate fraction. Due to the polar nature of the compounds in the active ethyl acetate fraction, the rotation locular counter-current chromatography (RLCC) method seemed to be suited for further separation.[6-8]

We describe in the present paper the efficient isolation of a bitter insect-antifeedant dihydrochalcone by RLCC, and the determination of its preferred conformation in solution by means of proton (^{1}H-) and carbon-13 (^{13}C-) nuclear magnetic resonance (NMR) studies.

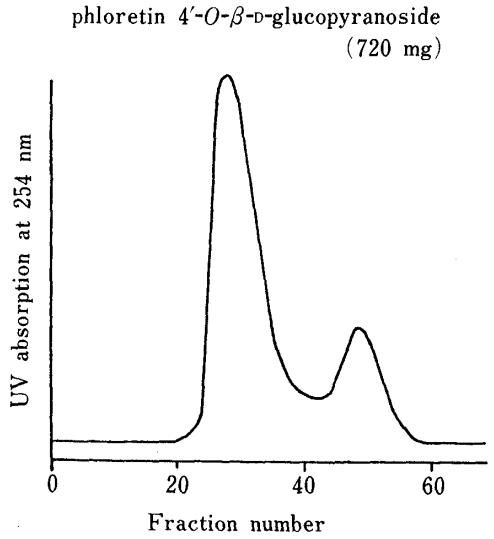


Fig. 1. RLCC of the MeOH Extract of *M. pumila* var. *dulcissima* (1.5 g) with $CHCl_3$–MeOH–H_2O (13:7:4, v/v) by the Ascending Method; 7 ml per Fraction; Detection at 254 nm

In order to avoid any loss of the active principles, the crude methanol extract was subjected to the RLCC separation, which was performed by the ascending method using chloroform–methanol–water (13:7:4, v/v) as a solvent system. The RLCC chromatogram monitored with an ultraviolet (UV) detector at 254 nm is shown in Fig. 1. The compound responsible for the antifeedant activity (**1**, mp 128—130 °C, M_r 436) was obtained by recrystallization following the RLCC separation. Spectroscopic data of **1** indicated the presence of a carbonyl group (ν 1610 cm^{-1}: δ_C 200.3) and a glucopyranoside (δ_C 60.5, 69.3, 73.0, 77.1, 76.5, 100.5; δ_H 4.96). Furthermore, 1,4-disubstituted (δ_C 114.5, 128.7; δ_H 7.03, 6.64) and 1,3,4,5-tetrasubstituted (δ_C 94.1, 96.6; δ_H 5.93, 6.15) aromatic moieties (ν 1580, 810 cm^{-1}: λ 220, 280 nm) were also indicated. Based on various physical and spectral evidence (mp, infrared (IR) spectrum, UV spectrum, chemical ionization mass spectrum (CI-MS), ^{1}H-, and ^{13}C-NMR), **1** was identified as the known dihydrochalcone, phloretin 4′-*O*-β-D-glucopyranoside.[3]

The antifeedant activity of **1** was ED_{50} 200 ppm against *S. graminum*.

Since the antifeedant activity and bitter taste of **1** were observed in solution, the conformation of **1** in solution could be of great importance for eliciting these activities. This led us to investigate the preferred conformation of **1** in solution by means of using a variety of ^{1}H- and ^{13}C-NMR experiments.

The ^{13}C-NMR spectrum showed two proton-bearing carbons at δ 96.6 and 94.1. These are assigned as A ring carbons of **1** (Fig. 2). Selective irradiation in the ^{1}H-NMR signal at δ 5.93 collapsed the large $^1J_{CH}$ (150 Hz) at δ 96.6 and irradiation of the ^{1}H-NMR signal at δ 6.15 collapsed the large $^1J_{CH}$ (150 Hz) at δ 94.1.

From the gated decoupling ^{13}C-NMR spectrum[9] (Fig. 3), the signal at δ 96.6 was seen to consist of a doublet of double doublets with one large $^1J_{CH}$ (150 Hz) and two smaller $^3J_{CH}$ (4.5 Hz) of almost equal size. The signal at δ 94.1 consisted of a double doublet with one large $^1J_{CH}$ (150 Hz) and one small $^3J_{CH}$ (4.5 Hz). Upon selective irradiation in the ^{1}H-NMR spectrum at δ 13.5, corresponding to an intramolecular hydrogen-bonded hydroxyl proton, one of the $^3J_{CH}$ couplings at δ 96.6 collapsed. This is consistent with the carbon giving the signal at δ 96.6 being adjacent to a hydrogen-bonded hydroxyl group. Although the carbon at δ 94.1 must also be adjacent to a hydroxyl proton and should be capable of having a $^3J_{CH}$ in theory, the absence of any observed coupling is probably due to rapid exchange of the hydroxyl proton. The above data are consistent with the suggested preferred conformation in solution as drawn in Fig. 2.

Upon irradiation of the anomeric proton at δ 4.96 (d, J=8 Hz), a 9% nuclear Overhauser effect (NOE) was seen at δ 6.15 with no enhancement at δ 5.93. This suggests that the plane of the A ring is perpendicular to that of the ring of the sugar moiety, with 5′-H in close proximity

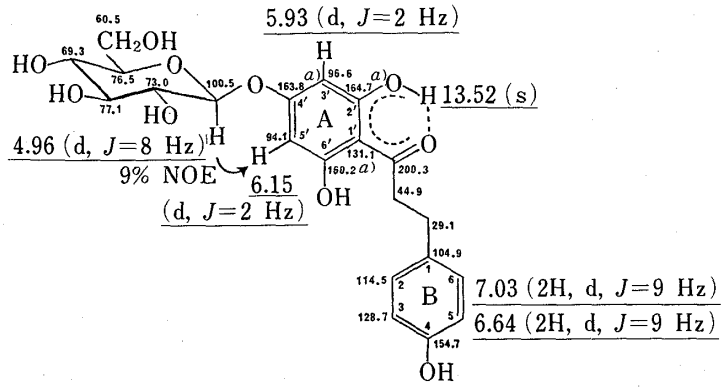


Fig. 2. ^{1}H-NMR (Underlined) and ^{13}C-NMR Assignments of **1** in DMSO-d_6

a) Assignments may have to be reversed.

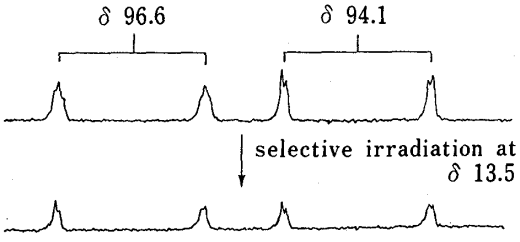
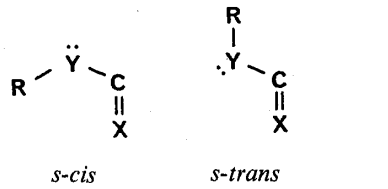


Fig. 3. Partial Gated Decoupled ^{13}C-NMR Spectrum with NOE of **1**



X=O, CH_2, CHR′, *etc.* Y=O, S, *etc.*

Fig. 4

to the anomeric proton.

Thus, the existence of a rigid conformation due to the hydrogen bonding of the 2′-OH and the carbonyl seems reasonable, but the reason why the anomeric proton is near the 5′-H is not clear. Molecular models show no obvious steric effects that would cause this preference. One possible rationalization is the effect of the lone pair of the glycoside bond oxygen hyperconjugating with the sp^2 orbitals of the A ring. It has been suggested that the reason why *s-cis* conformations[10] of vinyl ethers and other systems are favored over *s-trans* is because the hyperconjugative interaction of the hybrid lone pair, as shown in *ab initio* calculations,[11] is maximized for *s-cis* conformations (Fig. 4)[12]. A similar type of interaction in this instance would require more sp^2 character in the C(4′)–C(5′) bond than in the C(3′)–C(4′) bond. This is reasonable if the free hydroxyl oxygen at 6′-C is considered to be better able to add electron density to the ring than is the hydroxyl oxygen involved in the intramolecular hydrogen bond at 2′-C. This would place more electron density into the C(4′)–C(5′) bond than the C(3′)–C(4′) bond, although clearly calculations on this particular system are necessary to support this hypothesis.

Experimental

The melting point was determined on a Sybron Thermolyne Mp-12615 and is uncorrected. The IR spectrum was taken on a Perkin-Elmer 737B. The UV spectrum was recorded on a Hitachi 100-80. Specific optical rotation ($[\alpha]_D$) was measured with a Perkin-Elmer 241 and 241 MC apparatus. The MS was taken on a Finnigan 3300 instrument. ^{13}C- and ^{1}H-NMR spectra were obtained on a JEOL FX-400 spectrometer operating at 100 and 400 MHz, respectively.

Materials——Fresh leaves (111 g) of *M. pumila* var. *dulcissima* collected at Walnut Creek, California, in June, 1984, were extracted with MeOH for 1 d. After filtration, the filtrate was evaporated *in vacuo* to give 15.6 g of residue. The residue (2.0 g) was separated into fractions soluble in *n*-hexane (0.2 g), Et_2O (0.7 g), EtOAc (0.9 g), and H_2O (0.2 g).

RLCC Separation——The MeOH extract (1.5 g) was dissolved in 10 ml of the organic layer of $CHCl_3$–MeOH–H_2O (13:7:4, v/v), and injected into the RLCC apparatus (Tokyo Rikakikai Co., RLCC-A). The same solvent system and the ascending method were chosen for the separation. The eluates were collected in fractions of 7.0 ml per 30 min, and monitored with a UV detector at 254 nm (Pharmacia Fine Chemicals, single path monitor UV-1). The RLCC chromatogram is shown in Fig. 1.

Phloretin 4′-*O*-β-D-Glucopyranoside (1)——Colorless needles of mp 128—130 °C (recryst. from EtOAc); $[\alpha]_D^{25}$ −65.0° (c=0.5, EtOH). IR ν_{max}^{Nujol} cm^{-1}: 3500 (br OH), 1610 (CO), 1580, 810 (arom.). UV λ_{max}^{EtOH} nm (ε): 220 (27000), 280 (23000). CI-MS m/z: 437 ($M^+ + 1$), 419, 401, 325, 318, 299, 275, 257. ^{1}H-NMR (DMSO-d_6) δ: see Fig. 2. ^{13}C-NMR (DMSO-d_6) δ: see Fig. 2.

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References

1) I. Kubo and J. A. Klocke, "Plant Resistance to Insects: ACS Symposium Series 208," ed. by P. A. Hedin, American Chemical Society, Washington, D.C., 1983, p. 329.
2) D. L. Dreyer, J. C. Reese, and K. C. Jones, *J. Chem. Ecol.*, **21**, 273 (1981).

3) A. H. Williams, *J. Chem. Soc.*, **1961**, 4133.
4) C. D. Taper and P. S. Liu, *Can. J. Plant Sci.*, **49**, 97 (1969).
5) V. F. Verzilov, I. V. Plotnikova, and N. N. Bogomolova, *Fiziol. Rast.*, **19**, 674 (1972).
6) Y. Ito and R. L. Bowman, *J. Chromatogr. Sci.*, **8**, 315 (1970).
7) Y. Ito and R. L. Bowman, *Anal. Chem.*, **43**, 69A (1971).
8) I. Kubo and A. Matsumoto, *J. Agric. Food Chem.*, **32**, 687 (1984).
9) F. W. Wehrli and T. Wirthlin, "Interpretation of Carbon-13 NMR Spectra," Heyden, London, 1978, p. 83.
10) E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw Hill, New York, 1962, p. 331.
11) J. R. Larson, N. D. Epiotis, and F. Bernardi, *J. Am. Chem. Soc.*, **100**, 5713 (1978).
12) J. D. Mersh and J. K. M. Sanders, *Tetrahedron Lett.*, **40**, 4029 (1981).