UV Resonance Raman Detection of Artificial Sweetener in Soda Pop—Just for the Fun of It

MICHAEL LUDWIG and SANFORD A. ASHER*

Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

Index Headings: **Analysis of beverages; Raman spectroscopy.**

INTRODUCTION

Ultraviolet resonance Raman (UVRR) spectroscopy has recently demonstrated its potential as a powerful technique for the study of biomolecular structure[1,2] and for probing chromophores in complex systems.[3] UVRR investigations of proteins and peptides have demonstrated selective enhancement of the aromatic amino acid ring vibrations of small peptide chains and proteins.[2,4-6] UVRR is particularly useful for studying aromatic ring systems in complicated fuel, coal liquid, and petroleum mixtures.[7] An increase of up to six orders of magnitude in sensitivity can occur compared with that for normal Raman spectroscopy, because of the resonance phenomenon.

In this correspondance, the UVRR spectra of two soft drinks, Diet Coke and New Coke, are reported. UV excitation enhances phenylalanine ring modes in the artifical sweetener Nutrasweet in Diet Coke. This artifical sweetener is the dipeptide aspartame (L-phenylalanine, N-L-α-aspartyl-1 methyl ester; Nutrasweet® brand). Recently determined UVRR excitation profiles (a plot of the Raman cross section dependence on the excitation wavelength) of phenylalanine between 217 and 246 nm indicate that large Raman cross sections will occur as excitation approaches the 205-nm absorption band maximum.[2] The measured resonance Raman spectra of Diet Coke clearly shows scattering from the four resonantly enhanced phenylalanine ring vibrational modes. No other component of Diet Coke is enhanced with 220-nm excitation.

EXPERIMENTAL

Samples of Diet Coke and New Coke were obtained from a nearby vending machine and pumped directly from the can to the sample capillary. Phenylalanine was obtained from Sigma Chemical Company (St. Louis, MO). The Raman spectra of the soft drinks and phenylalanine were measured in a flowing closed cycle recirculating stream in which the solutions were pumped through a 1.0-mm-internal-diameter Suprasil quartz capillary tube. The scattered light was collected at 90°. The Raman spectrometer has been described in detail elsewhere.[8,9] The 12-fl.-oz (354 mL) samples of Diet Coke and New Coke were irradiated for 15 min with the use of energies of 0.5 mJ/pulse at a 20 Hz repetition rate.

RESULTS AND DISCUSSION

Figure 1 shows the absorption spectra of Diet Coke and New Coke. Phenylalanine has absorption maxima at 205 nm (ϵ = 9600 M^{-1} cm^{-1}) and 258 nm (ϵ = 190 M^{-1} cm^{-1}).[10] The UV absorption spectrum of Diet Coke shows significant absorption at wavelengths below 220 nm due, at least in part, to aspartame. In contrast, New Coke absorbs significantly less in this frequency region.

Excitation of phenylalanine far from resonance results in a normal Raman spectra showing peaks derived from vibrations of both the aromatic side chain and the α-aminocarboxyl group. Raman spectra measured in resonance, however, show enhancement only of vibrations localized on the aromatic side chain of phenylalanine.[4]

Figure 2 shows the resonance Raman spectra of a 0.005-M aqueous solution of phenylalanine (pH 11.5), Diet Coke, and New Coke, and the difference spectrum between Diet and New Coke, each obtained with 220-nm excitation. The shaded peak at 932 cm^{-1} in the phenylalanine spectrum is due to sodium perchlorate, which is used as an internal intensity standard. The Raman peaks of phenylalanine are assigned by analogy to spectra of benzene and toluene. The symmetric ring stretching mode (ν_1) occurs at 790 cm^{-1} in phenylalanine and is not strongly enhanced in resonance with the 205-nm electronic transition. The 1006 cm^{-1} band derives from a mode resembling the ν_{12} benzene ring vibration. The 1182 cm^{-1} band is an in-plane C-H bending mode (ν_{9a}).

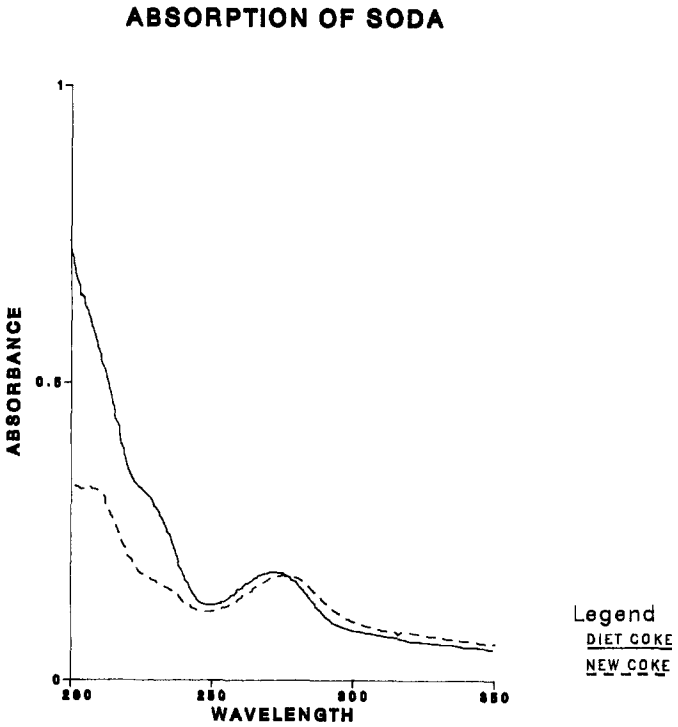

FIG. 1. Absorption spectra of Diet Coke and New Coke. Each sample was diluted in water by a factor of 400. The absorption maxima of phenylalanine occur at 205 nm (ϵ = 9600 M^{-1} cm^{-1}) and 258 nm (ϵ = 190 M^{-1} cm^{-1}).

Received 21 July 1986.
* Author to whom correspondence should be sent.

0003-7028/87/4102-0331$2.00/0

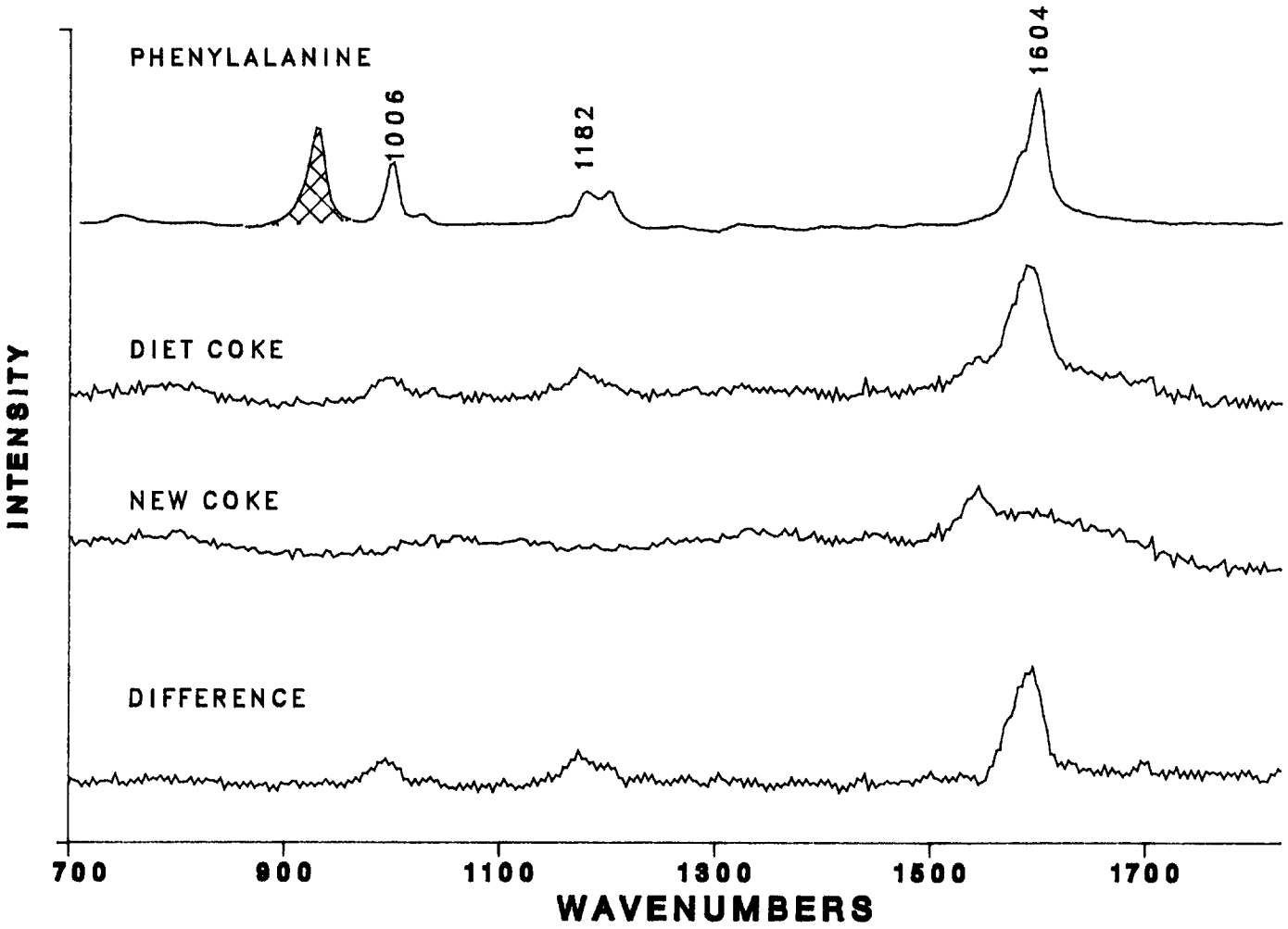

FIG. 2. Resonance Raman spectra excited at 220 nm: aqueous solutions of phenylalanine (0.005 M) containing 1.0 M sodium perchlorate (932 cm^{-1} band of perchlorate is shaded), power = 0.35 mJ/pulse; Diet Coke, power = 0.50 mJ/pulse; New Coke, power = 0.50 mJ/pulse; Raman difference spectrum (Diet Coke minus New Coke).

The band at 1604 cm^{-1}, actually an unresolved doublet, derives from the benzene ν_8 vibration. The degeneracy of the ν_8 (e_{2g}) benzene vibration is removed by the ring substituent. The ν_{8a} and ν_{8b} bands are close in frequency and are not resolved in the spectra.

Diet Coke is 0.15 mM in aspartame.[11] The UVRR spectrum of Diet Coke shows four resonantly enhanced phenylalanine peaks. In contrast, the Raman spectrum of New Coke at 220 nm shows no resonantly enhanced phenylalanine bands, since no aspartame is present. The Raman difference spectrum between the spectra of Diet Coke and New Coke is almost identical to the phenylalanine resonance Raman spectrum. Differences in frequency and intensity of the bands observed presumably occur because of differing molecular environments of the aromatic ring between monomeric phenylalanine and the dipeptide aspartame in the Diet Coke mixture.

These experiments illustrate the ability of UV resonance Raman spectroscopy to selectively examine individual components within a complex matrix. Aspartame is easily studied in a sample of Diet Coke. In addition to the unique selectivity available from resonance enhancement with excitation in the phenylalanine ring $\pi \rightarrow \pi^*$ transition, it should also be noted that even in the UV, Raman scattering from the water (55 Molar) is weak.

ACKNOWLEDGMENTS

We gratefully acknowledge partial support of this work from NIH Grant 1-RO1GM30741-04. Sanford A. Asher is an Established Investigator of the American Heart Association; this work was done during the tenure of an Established Investigatorship of the American Heart Association and with funds contributed in part by the American Heart Association, Pennsylvania affiliate.

1. C. M. Jones, T. Naim, M. Ludwig, J. Murtaugh, P. F. Flaugh, J. M. Dudik, C. R. Johnson, and S. A. Asher, Trends Anal. Chem. **4,** 75 (1985).
2. S. A. Asher, M. Ludwig, and C. R. Johnson, J. Am. Chem. Soc. **108,** 3186 (1986).
3. C. R. Johnson and S. A. Asher, Anal. Chem. **56,** 2258 (1984).
4. C. R. Johnson, M. Ludwig, S. E. O'Donnell, and S. A. Asher, J. Am. Chem. Soc. **106,** 5008 (1984).
5. R. P. Rava and T. G. Spiro, Biochemistry **24,** 1861 (1985).
6. R. A. Copeland, S. Dasgupta, and T. G. Spiro, J. Am. Chem. Soc. **107,** 3370 (1985).
7. S. A. Asher and C. M. Jones, Am. Chem. Soc. Fuel Science Reprints **31,** 170 (1986).
8. S. A. Asher, C. R. Johnson, and J. L. Murtaugh, Rev. Sci. Instru. **54,** 1657 (1983).
9. S. A. Asher, Appl. Spectrosc. **38,** 276 (1984).
10. I. Weinryb and R. F. Steiner, in *Excited States of Proteins and Nucleic Acids,* R. F. Steiner and I. Weinryb, Eds. (Plenum Press, New York, 1971), p. 277.
11. Coca-Cola Company, personal communication.