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Short communication

Identification of Genistein and Biochanin A by THz (far-infrared) vibrational spectra

Yuping Yang^{a,b}, S. Sree Harsha^a, Alisha J. Shutler^a, Daniel R. Grischkowsky^{a,*}^a School of Electrical and Computer Engineering, Oklahoma State University, Stillwater, OK 74078, USA^b School of Science, Minzu University of China, Beijing 100081, China

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ABSTRACT

The temperature dependent vibrational spectra of Genistein and Biochanin A, major components in Mongolian medicines *Agi*, in the range of 0.5–4.5 THz (16.7–150 cm⁻¹) are presented for the first time. Over the temperature range from 295 to 77 K, 12 highly resolved spectral features for the Genistein and 13 features for Biochanin A were measured by THz-TDS and display strong linewidth narrowing and frequency blue-shift with cooling. Such narrow-line THz fingerprint spectra provide a rapid, nondestructive and reliable method for the identification of these Chinese traditional medicines.

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1. Introduction

Historically, Chinese traditional medicines have played an important role in clinical therapy for thousands of years. Recently, the pharmaceutical value of Mongolian medicines like *Artemisia frigida wild* (*Agi*) has increased owing to their ability to inhibit tumor growth, estrogen production and other effects. Unlike synthetic drugs, herbal medicine is a complicated system of mixtures. The identification and quality determination of these botanical drugs using modern analytical techniques, such as high performance liquid chromatography, gas chromatography, gas chromatography–mass spectrometry and thin layer chromatography, are quite difficult due to the time-consuming and laborious pre-preparation of the samples. In recent years, molecular spectroscopic techniques, including Raman, and IR have been applied for rapid, simple and non-destructive identification and quantitative analysis [1,2]. However, these measurements do not reach the far-infrared (FIR) spectral region, which has been shown to contain a wealth of information regarding low-frequency vibrational motion of molecules.

As an attractive and unique spectroscopic technique in the FIR range, Terahertz time-domain spectroscopy (THz-TDS), as shown in Fig. 1, can reveal rich structural information of molecules, by

measuring the frequencies of the inter- or intra-molecular vibrational modes. Recently, Chen et al. [3] applied THz-TDS to identify two kinds of similar medicine by using the Support Vector Machine (SVM) method. Besides that, THz-TDS can be used to investigate the interaction with biomolecular and pharmaceutical mechanisms. For example, Wang et al. [4] have studied the THz spectra of 1,4-naphthoquinone and its four derivatives. However, without a complete compilation of the characteristic THz (far-infrared) fingerprint spectra for these medicines, the present use of THz-TDS for identifying and studying many Chinese drugs is not possible. Also, due to homogenous and inhomogenous line broadening mechanisms [5], most of absorption features of major components, which are only slightly different in structure and are characterized at room temperature, tend to be rather broad and overlapping, and are difficult to distinguish.

In this paper, Genistein (5,7,4'-trihydroxyisoflavone, GEN) and Biochanin A (5,7-dihydroxy-4'-methoxyisoflavone, BCA), major components in Mongolian medicines *Agi*, are characterized in the Terahertz range using the standard sample pellet technique and the high-resolution THz-TDS technique over the temperature range 77–295 K.

Previously, the molecular interaction of GEN and cyclodextrin (CD) has been studied by UV-vis, FTIR-ATR and Raman spectroscopic methods [6–8]. These studies focused on the enhancement of aqueous solubility and the molecular inclusion between isoflavones and CDs. The changes in host–guest interactions have been evidenced by monitoring the guest molecule's bands and their

* Corresponding author. Tel.: +1 405 744 6622; fax: +1 405 744 9198.
E-mail address: daniel.grischkowsky@okstate.edu (D.R. Grischkowsky).

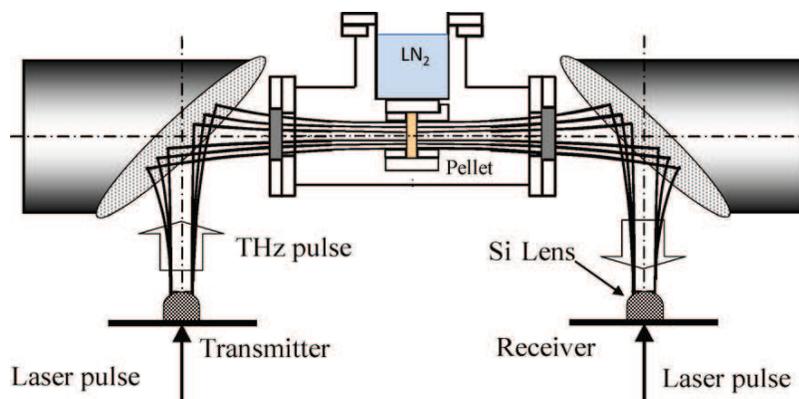


Fig. 1. Low-temperature THz-TDS apparatus.

vibrational spectra. So far, however, far-infrared (FIR) spectra of pure GEN and BCA below 150 cm^{-1} (4.5 THz) have not been investigated.

In our work, a number of well-resolved absorption lines for both GEN and BCA were measured in the frequency range $0.2\text{--}4.5\text{ THz}$ for the first time and display strong linewidth narrowing and frequency blue-shift with cooling. The line narrowing reveals many of the underlying vibrational resonances of GEN and BCA and thereby provides a new opportunity to assign individual lines to their specific vibrational motions.

2. Experimental

2.1. Sample preparation

Both Genistein (>97% purity) and Biochanin A (>98% purity) samples were purchased from Alfa Aesar Company and used without further purification. GEN and BCA have molecular weights of 270 and 284, respectively. The molecular structures of GEN and BCA are schematically shown in Figs. 2 and 3. It can be seen that the A and C rings of GEN are structurally identical to those of BCA, but have the only difference in the O-groups at position C-4' of ring B. The sample pellets were prepared by grinding and mixing 25 mg of GEN or BCA powder with 330 mg of polyethylene (PE) powder, and then the uniform mixture was compressed into a 1 cm diameter, 2 mm thick pellet, using a Carver hydraulic press up to a maximum pressure of 11 metric tons. For comparison, we also prepared

the reference pellet consisting of pure PE powder with identical PE mass (330 mg).

2.2. THz-TDS setup

A standard THz-TDS setup based on photoconductive switches was used for sample characterization (see Fig. 1) [9]. THz radiation is generated and detected using photoconductive switches driven by 10 mW optical pulse trains from an 800 nm, 80 fs, 100 MHz mode locked Ti: sapphire femtosecond laser. The emitted THz radiation is collimated by a high-resistivity Si lens and a parabolic mirror. A measurement with a frequency resolution of 1 GHz (0.033 cm^{-1}) and a time domain signal-to-noise (S/N) ratio of 10,000 can be achieved with this system. The entire system is located in an airtight enclosure to overcome the effects of water vapor absorption on the THz beams. The system also incorporates a LN_2 cryostat coupled to a vacuum chamber with Si windows to facilitate low temperature measurements.

Typically, six reference pulses transmitted through the pure PE pellet and six sample pulses transmitted through the GEN or BCA pellet were measured and averaged. For all THz reference and sample pulses, the measured temporal scan length was 33.33 ps, corresponding to a frequency resolution of 30 GHz (1.00 cm^{-1}). To improve the frequency resolution, the measured pulses in the time domain were zero-padded by a factor 4 to a total time duration of 133.33 ps. Then, the reference and sample amplitude spectra were obtained from their complex Fourier transforms, $A(\omega)_R$ and $A(\omega)_S$,

Table 1
Measured line-center frequencies and uncertainties for GEN and BCA.

Genistein		Biochanin A	
295 K	77 K	295 K	77 K
1.443 ± 0.001 (0.060)	1.531 ± 0.001 (0.042)	1.121 ± 0.001 (0.058)	1.164 ± 0.002 (0.052)
1.61 (sh)	1.677 ± 0.001 (0.034)	–	1.44 (w)
1.731 ± 0.001 (0.152)	1.791 ± 0.001 (0.040)	1.572 ± 0.002 (0.068)	1.638 ± 0.002 (0.058)
1.80 (sh)	1.950 ± 0.001 (0.073)	1.706 ± 0.003 (0.104)	1.76 (w)
2.014 ± 0.002 (0.119)	2.139 ± 0.001 (0.097)	–	1.86 (w)
–	2.32 (w)	2.08 (w)	2.02 (w)
2.45 (w)	2.54 (w)	2.270 ± 0.003 (0.280)	2.373 ± 0.007 (0.168)
–	2.64 (w)	2.607 ± 0.007 (0.143)	2.709 ± 0.007 (0.087)
2.673 ± 0.002 (0.120)	2.819 ± 0.002 (0.060)	–	2.86 (w)
–	2.886 ± 0.002 (sh)	3.090 (w)	3.222 ± 0.007
3.105 ± 0.005 (0.206)	3.312 ± 0.002 (0.113)	3.303 (w)	3.457 ± 0.007
3.808 ± 0.006 (0.353)	4.013 ± 0.015 (0.122)	–	3.89 (sh)
–	–	3.837 ± 0.025 (0.154)	3.991 ± 0.007

Note: (1) (sh) corresponds to a weak peak evident on a shoulder of an absorption line; (w) corresponds to a weak peak; (2) FWHM linewidths for absorbance are given in parenthesis; (3) all values are given in THz ($1\text{ THz} = 33.33\text{ cm}^{-1}$).

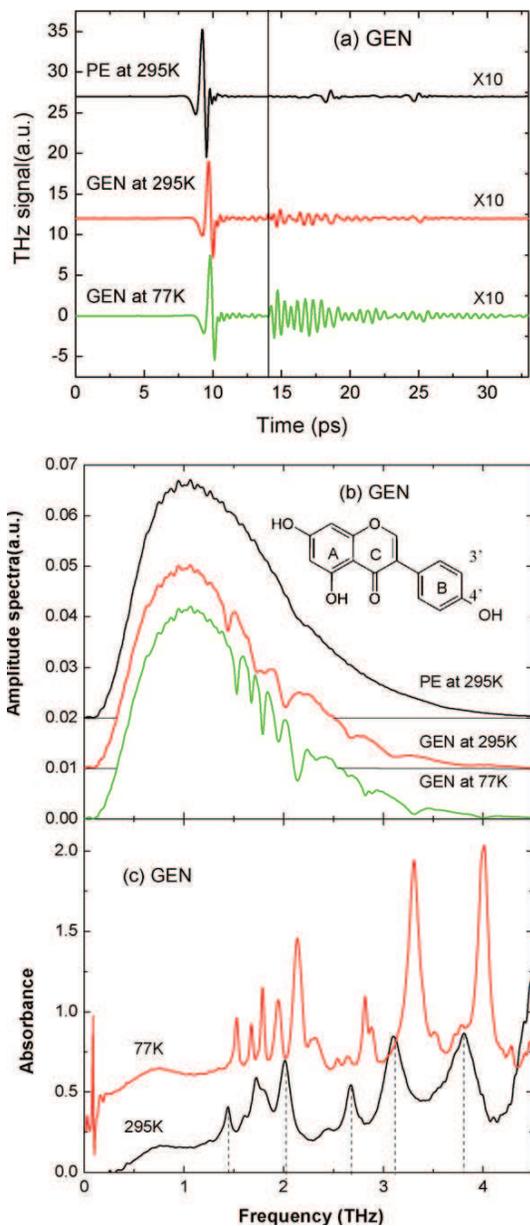


Fig. 2. (a) Measured transmitted THz pulses. Note for delay times greater than 14 ps the pulse amplitude has been multiplied by 10. (b) Corresponding amplitude spectra and (c) amplitude absorbance spectra of Genistein at 77 and 295 K (1 THz = 33.33 cm⁻¹).

respectively. The amplitude absorbance shown in Figs. 2 and 3 is given by,

$$\text{absorbance} = -\ln \left[\frac{A(\omega)_S}{A(\omega)_R} \right].$$

3. Results and discussion

3.1. Experimental data

The transmitted THz pulses through the GEN pellet and the reference PE pellet, are shown in Fig. 2(a). The time window for the pulses is limited by a reflection to be 33.33 ps long. However, this scan length is long enough to measure the temporal ringing (which

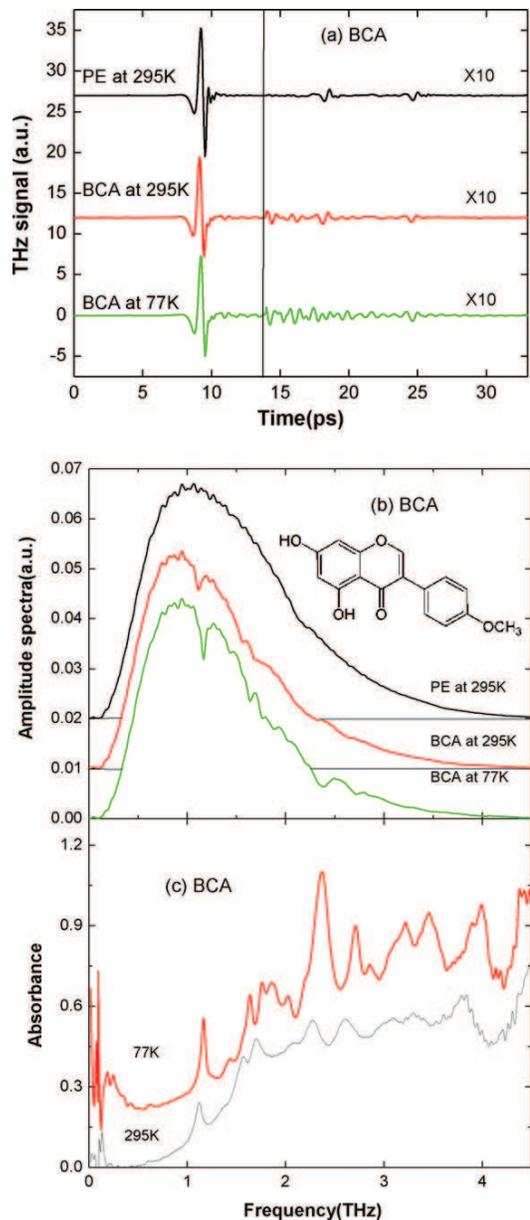


Fig. 3. (a) Measured transmitted THz pulses. Note for delay times greater than 14 ps the pulse amplitude has been multiplied by 10. (b) Corresponding amplitude spectra and (c) amplitude absorbance spectra of Biochanin A at 77 and 295 K (1 THz = 33.33 cm⁻¹).

directly corresponds to the spectral features), because oscillation dies down quickly, even for the longer, more pronounced ringing observed for measurements at 77 K. This can be seen in Fig. 2(a), where the signals past 14 ps have been multiplied by 10.

Longer ringing corresponds to sharper and deeper spectral features, as can be seen in Fig. 2(b), and sharper and stronger absorbance lines, as shown in Fig. 2(c). At room temperature, relatively weak and broad absorption features are observed. As the GEN pellet is cooled to 77 K, the broad absorption features seen at 295 K undergo a pronounced sharpening, and many additional absorption features begin to appear. Also, the vibrational lines shift toward higher frequencies. Similar measurements for the BCA pellet are presented in Fig. 3. Unlike GEN, BCA has a continuous, broadband

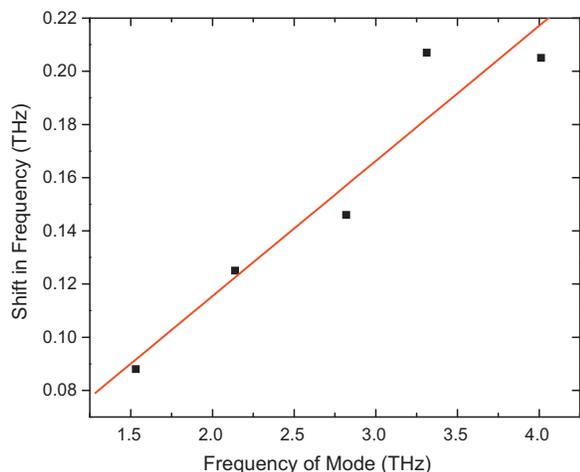


Fig. 4. The temperature-dependent frequency shifts (77–295 K) are plotted vs mode frequency for GEN. The five most intense bands (taken from Table 1) are used in the analysis (1 THz = 33.33 cm⁻¹).

absorption that increases with frequency. The measured absorption line centers are listed in Table 1 for GEN and BCA, respectively.

3.2. Comparison of GEN and BCA

Due to similar chemical structures of Genistein and Biochanin A, some absorption bands of BCA, around 1.71, 2.61 and 3.84 THz (57.0, 120.0 and 128.0 cm⁻¹) at 295 K, are similar to those of GEN, but weaker in intensity. However, at frequencies around 1.11, 1.44, 2.27 and 3.10 THz (37.0, 48.0, 75.7 and 103.3 cm⁻¹) at 295 K, the spectra of Genistein and Biochanin A are quite different in both absorption band positions and their relative intensities. This is reflected in the radically different spectral THz signatures for each molecule due to only a minor difference in their molecular structures. At 77 K, the much narrower lines make the THz absorption spectra of GEN and BCA more distinguishable.

3.3. Temperature dependent THz spectra for GEN

As the temperature is reduced from room temperature to 77 K, the linewidths decrease, and most of the observed peaks for both samples shift toward higher frequencies. Table 1 shows that the FWHM linewidths and temperature shifts of the vibrational frequencies are quite different for different transitions. We quantified the blue-shift effect by plotting the temperature-dependent frequency shift vs mode frequency for the five most intense bands of GEN, taken from Table 1, and indicated in Fig. 2(c) by the dashed vertical lines. The result in Fig. 4 shows that the frequency shifts of the peak positions increase linearly with frequency, indicating that the physical origin of the temperature shift is the same for all of these transitions.

Shifts in peak position and line broadening have been observed in the THz vibrational spectra of many micro-crystalline organic molecules [10–15]. The peak shifts toward higher frequencies can be attributed to compression of the crystalline lattice upon cooling, which results in a steeper intermolecular potential and an anharmonicity of the vibrational potentials, thereby causing the spacing between adjacent energy levels to decrease for the higher energy vibrational states [5]. For low-frequency vibrational modes (below 6 THz) at 295 K, the ground state vibrational levels are significantly depopulated by the thermal distribution. As a result, the envelope of transitions from different initial states has an inhomogeneous line

shape and a broadened band. In Fig. 2, all of the five most intense peaks, at 1.443, 2.014, 2.673, 3.105 and 3.808 THz (48.1, 67.1, 89.1, 103.5, and 126.9 cm⁻¹), show an asymmetric line shape at 295 K, indicating a large anharmonicity of the vibrational potential, which can explain the temperature dependence of the frequency shift observed in our experiments.

In addition, as observed, the distribution of the states should be narrower at lower temperature. Not only can such narrow lines result in a more precise measurement of the vibrational frequency, but they also can make the THz absorption spectra of different molecules more distinguishable, which lead to more accurate “spectral fingerprints” for identification purposes.

4. Conclusion

In this work, we have used THz-TDS to measure the temperature-dependent spectral signatures for both Genistein and Biochanin A, important anti-carcinogenic compounds that are commonly found in soy-rich diets. We find that as the temperature is reduced, the observed absorption bands for both GEN and BCA resolve into narrower peaks and shift toward higher frequencies, due to the cooling thermal distribution of the molecules and the anharmonicity of the vibrational potentials. In addition, when these narrow spectral fingerprints are used in conjunction with solid state theory, the inter- and intra-molecular vibrations of Genistein and Biochanin A may be more fully understood.

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References

- [1] J. Mao, J. Xu, Discrimination of herbal medicines by molecular spectroscopy and chemical pattern recognition, *Spectrochim. Acta Part A* 65 (2006) 497–500.
- [2] Y.A. Woo, H.J. Kim, J.H. Cho, H. Chung, Discrimination of herbal medicines according to geographical origin with neat infrared reflectance spectroscopy and pattern recognition techniques, *J. Pharm. Biomed. Anal.* 21 (1999) 407–412.
- [3] Y.J. Chen, Y.Y. Liu, G.Z. Zhao, W.N. Wang, F.L. Li, Chinese traditional medicine recognition by support vector machine (SVM) terahertz spectrum, *Spectrosc. Spect. Anal.* 29 (2009) 2346–2350.
- [4] W. Wang, H. Li, X. Luo, X. Zeng, THz spectra of 1,4-naphthoquinones and its four derivatives, *Proc. SPIE* 6840 (2007), 6840001-7.
- [5] A. Xie, Q. He, L. Miller, B. Sclavi, M.R. Chance, Low frequency vibrations of amino acid homopolymers observed by synchrotron far-IR absorption spectroscopy: excited state effects dominate the temperature dependence of the spectra, *Biopolymers* 49 (1999) 591–603.
- [6] Á.E. Daruházi, L. Szente, B. Balogh, P. Mátyus, S. Béni, M. Takács, A. Gergely, P. Horváth, É. Szöke, É. Lemberkovic, Utility of cyclodextrins in the formulation of genistein. Part 1. Preparation and physicochemical properties of genistein complexes with native cyclodextrins, *J. Pharm. Biomed. Anal.* 48 (2008) 636–640.
- [7] V. Crupi, R. Ficarra, M. Guardo, D. Majolino, R. Stancanelli, V. Venuti, UV-vis and FTIR-ATR spectroscopic techniques to study the inclusion complexes of genistein with β -cyclodextrins, *J. Pharm. Biomed. Anal.* 44 (2007) 110–117.
- [8] V. Crupi, D. Majolino, A. Paciaroni, B. Rossi, R. Stancanelli, V. Venuti, G. Viliiani, The effect of hydrogen bond on the vibrational dynamics of genistein free and complexed with β -cyclodextrins, *J. Raman Spectrosc.* 41 (2010) 764–770.
- [9] D. Grischkowsky, S. Keiding, M. van Exter, Ch. Fattinger, Far-infrared time-domain spectroscopy with terahertz beams of dielectrics and semiconductors, *J. Opt. Soc. Am. B* 7 (1990) 2006–2015.
- [10] Y.C. Shen, P.C. Upadhy, E.H. Linfield, A.G. Davies, Temperature-dependent low-frequency vibrational spectra of purine and adenine, *Appl. Phys. Lett.* 82 (2003) 2350–2352.
- [11] N. Laman, S.S. Harsha, D. Grischkowsky, J.S. Melinger, 7 GHz resolution waveguide THz spectroscopy of explosives related solids showing new features, *Opt. Express* 16 (2008) 4094–4105.
- [12] N. Laman, S.S. Harsha, D. Grischkowsky, Narrow-line waveguide Terahertz time-domain spectroscopy of Aspirin and Aspirin precursor, *Appl. Spectrosc.* 62 (2008) 319–326.

- [13] S.S. Harsha, D. Grischkowsky, THz (far infrared) characterization of Tris (hydroxymethyl) aminomethane (TRIS) using high resolution waveguide THz-TDS, *J. Phys. Chem. A* 114 (2010) 3489–3494.
- [14] J.S. Melinger, S. Sree Harsha, N. Laman, D. Grischkowsky, Temperature dependent characterization of THz vibrations of explosives and related threat materials, *Opt. Express* 18 (2010) 27238–27250.
- [15] H. Hoshina, Y. Morisawa, H. Sato, H. Minamide, I. Noda, Y. Ozaki, C. Otani, Polarization and temperature dependent spectra of poly(3-hydroxyalkanoate)s measured at terahertz frequencies, *Phys. Chem. Chem. Phys.* 13 (2011) 9173–9179.