New Sources of Sucrose as a Solid Plant Exudate[1]

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Abstract: We report two new natural sources of sucrose as a pure, solid exudate from *Dermatophyllum secundiflorum* (Texas mountain laurel) and *Viburnum rhytidophyllum* (leatherleaf or wrinkled viburnum). These exudates have been characterized by nuclear magnetic resonance spectroscopy and compared to authentic samples of sucrose from natural sources (sugar cane, *Saccharum officinarum* and sugar maple, *Acer saccharum*) and from commercial sources (refined sugar). Spectra were recorded both of the carbon-13 nuclei in the solid state and of the hydrogen nuclei in solution.

Key Words: carbohydrates, exudation, nuclear magnetic resonance spectroscopy, sugar cane, sucrose, sugar maple, Texas mountain laurel, wrinkled viburnum

Sucrose is the chemical name for the historically most common sweetener, called white or table sugar, which is produced and commercialized in a granulated and crystallized form. The primary source of sucrose is sugar cane, which comprises several cultivated species of the genus *Saccharum*, including primarily *S. officinarum*. The wild species probably originated in New Guinea and migrated through Southeast Asia to India, where refinement was developed some two thousand years ago (Mintz 1986, Sharpe 1998, Adas 2001, Paterson et al 2012). Sugar cane followed the Arab advance across Southwest Asia to the Mediterranean and reached Spain via Africa by the early eighth century, where a robust European sugar industry developed. Sugar cane is shredded and crushed to produce a juice containing 15-20% sucrose, which is purified and crystallized in a series of steps. The root of the sugar beet (*Beta vulgaris*) is a secondary source of refined sucrose.

Sucrose is a member of a chemical family of generally sweet-tasting molecules jointly called sugars, carbohydrates, or saccharides, which contain three or more carbon atoms but most commonly six carbons (hexoses, the suffix *–ose* signifying a sugar). Sugars that contain a single such unit are called

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monosaccharides, and sugars containing two units are called disaccharides. All monosaccharide hexoses have the formula of $C_6H_{12}O_6$, which rearranged can be expressed as $[C(H_2O)]_6$, from which evolved the early misnomer hydrated carbon or carbohydrate. The terms sugar, sucrose, and saccharide came from Sanskrit (*śarkara*) via Persian (*shaker*) and French (*sucre*) to English (Galloway 1989). Early molecular formulations portrayed sugars as a linear array of carbon functions traditionally written beginning with an aldehyde (CHO—), continuing with secondary alcohols (>CHOH), and terminating with a primary alcohol (—CH_2OH): CHO—$[CH(OH)]_n$—CH_2OH (n = 4 for a hexose). Such sugars have been called aldoses from the aldehyde functionality, although the carbonyl group also may be found internally as a ketone in ketoses.

Each of the carbon atoms of the tertiary alcohol is chiral, that is, there are four different groups attached to the carbon. The presence of multiple chiral centers results in a given formula having several different stereochemical forms. The structures of sugars in all their stereochemical modifications famously was explicated by Emil Fischer in the last years of the nineteenth century, for which he was awarded the 1902 Nobel Prize in Chemistry (the second, after van 't Hoff in 1901). Among the many complexities of sugar structures, Fischer discovered that the aldehyde at C1 reacts with the tertiary alcohol at C5 to form a ring, which is the predominate form in equilibrium with the open-chain form. As the result of ring formation, the C1 aldehydic atom that forms the connection with the C5 alcohol oxygen becomes bonded to two oxygen atoms, whereas all other atoms are bonded to a single oxygen atom. Consequently, the C1 carbon has special properties and is referred to as the *anomeric carbon*. Examples of monosaccharidic hexoses include glucose and maltose. Fructose also is a hexose but in the open form contains a ketone at C2, which reacts with the alcohol oxygen on C5 to form a five-membered ring. Sucrose, however, is a disaccharide consisting of one glucose and one fructose unit linked by an oxygen atom between the C1 glucose anomeric carbon and the C2 fructose anomeric carbon, as in Figure 1. The glucose ring is on the left in the figure, portrayed in the so-called chair conformation, and the fructose ring is on the right, portrayed in a slightly incorrect planar conformation (the actual five-membered ring is not fully planar to relieve atom-atom repulsions present in the planar form).

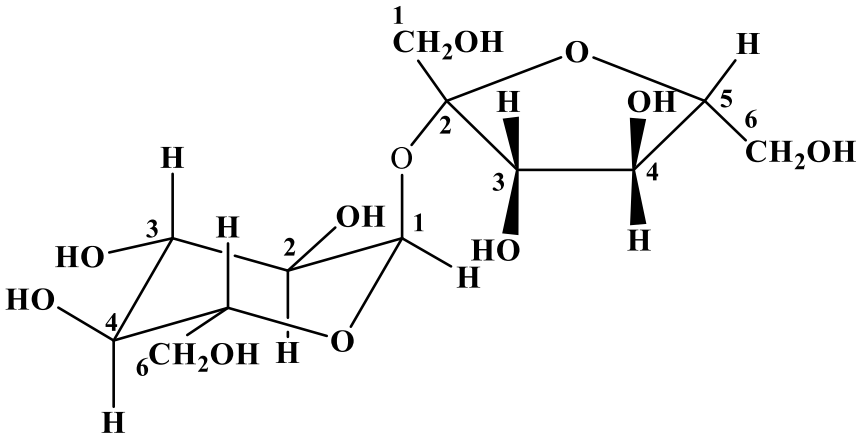

Figure 1. The molecular structure of sucrose.

Although the common commercial sources of sucrose are *S. officinarum* (sugar cane) and *B. vulgaris* (sugar beet), there are other sources. Sucrose and other sugars are found commonly in fruits, roots, and nectars of numerous plants, which are not commercialized because the plant is rare, the levels of sucrose are too low, or an extraction process is not practical. Minor amounts of sucrose have been commercialized from sorghum (*Sorghum vulgare*) and sugar maple (*Acer saccharum*). Date palms (*Phoenix dactylifera*) contain so-called invert sugar, composed of nearly equal amounts of glucose and fructose rather than sucrose itself *(*Yasawy 2016). Artificial sweeteners (aspartame, cyclamate, saccharin, and sucralose) generally are sweet chemicals based on structures other than carbohydrates, although sucralose is a chlorinated carbohydrate (O'Brien-Nabors 2011).

For the past 25 years we have been acquiring plant exudates on a worldwide basis and carrying out molecular analysis by nuclear magnetic resonance (NMR) spectroscopy (Lambert et al. 2007, 2008). We have had access to museum collections and botanical gardens throughout North America. In this fashion we have acquired and characterized over 1500 plant exudates. There are three principal molecular classes of exudates: resins composed largely of terpene components, gums containing long carbohydrate chains, and phenolics with aromatic rings predominating (Lambert et al. 2021). Less frequently encountered molecular classes of exudates include waxes, gum resins, xanthates, and balsamics (Lambert et al. 2022). All these molecular groups have large molecular weights, representing mixtures of oligomers and polymers, but few are specific molecules. In the process of obtaining and analyzing these materials, we adventitiously have identified five exudates to be pure sucrose. Two of these were

from the expected sources, sugar cane *S. officinarum*, and one was from the sugar maple tree, *A. saccharum*. In addition, however, two sucrose samples came from unexpected sources: *Viburnum rhytidophyllum* and *Dermatophyllum secundiflorum*. This study surveys all these natural sources of sucrose.

Methods

The five samples in this study came from a variety of sources. Samples 1462 and 1463 of *S. officinarum* (family Poaceae, clade monocot, common sugar cane) were obtained from the Materia Medica Collection at the National Museum of American History by author JASB. Both were labeled "rock candy," which probably implies that they had been processed prior to acquisition. Sample 1462 was orangish yellow and carried the acquisition number 51,973, and sample 1463 was light brownish yellow and carried the acquisition number 51,972. The solid, creamish white exudate from sugar maple (sample 2118, *A. saccharum*, family Sapindaceae, clade eurosid I) was harvested by author JASB and S. Shaffer on the grounds of St. Paul's Catholic Church, Springettsbury Township, York Co., PA. This material was allowed to dry but otherwise was examined without any processing. The other two samples had not been processed from their natural state, other than powdering. The very dark sample 1500 of *Viburnum rhytidophyllum* (family Adoxaceae, clade euasterid II, leatherleaf or wrinkled viburnum) was collected by author JASB with permission from the Bernheim Arboretum and Forest, Clermont, KY, acquisition number NA49608. The light orange sample 1592 from *Dermatophyllum secundiflorum* (family Fabaceae, clade eurosid I, Texas mountain laurel) was provided by Emily Rockey, Curator of Horticulture, Tucson Botanical Gardens, Tucson, AZ. The collectors were unaware that the harvested exudates of the last two materials were sucrose. The appearance of exudates as they occur in Nature provides little information about their molecular makeup. Thus, another exudate sample of *S. officinarum* (948) proved to be a wax (Lambert et al. 2022), and another sample of *A. saccharum* (1828) was a gum resin. Only by molecular analysis can the composition of the exudate be revealed.

NMR spectra were taken in four modes. Carbon (^{13}C) spectra were recorded with full decoupling (removal of interactions with hydrogen atoms, referred to herein as protons) and with interrupted decoupling (also called dipolar dephasing), a technique to select for quaternary carbons (those lacking C—H bonds), although some rapidly moving carbons also appear in the spectrum. Both the normal one-dimensional (1D) proton (^{1}H) was recorded and the two-dimensional COSY (correlation spectroscopy) spectrum. Because the samples were insoluble in $CDCl_3$, we examined them in deuterated dimethyl sulfoxide [$(CD_3)_2SO$].

Solid state ^{13}C data were recorded on a 400 MHz Varian NMR System with a 5-mm T3 PENCIL probe or on a 400 MHz Bruker Avance III HD NMR

Spectrometer with a 4-mm HX probe, both at Northwestern University. The magic angle spinning rate was set to 5000 Hz. The cross polarization (CP) pulse sequence was used for normal proton decoupling on both spectrometers. For interrupted decoupling (dipolar dephasing), a 50-μs (Varian) or a 48-μs (Bruker) delay was applied in the ^{1}H channel just before the 180° pulse in the ^{13}C channel. We used adamantane (Varian) or glycine (Bruker) to adjust the Hartmann-Hahn matching condition for normal CP experiments and to adjust the observation pulse and the delay time for dipolar dephasing. A typical parameter set was as follows: spectrum frequency 100.544 MHz (Varian) or 100.524 MHz (Bruker), spectral width 296 ppm, pulse width 3.4 μs for the 90° pulse for both ^{1}H and ^{13}C (Varian) or 2.5 μs for ^{1}H and 4.0 μs for ^{13}C (Bruker), pre-delay time 5 s, contact time 5 ms, acquisition time 50 ms, scan number 256, carrier frequency 110 ppm, and a ramped pulse with 83 Watts used in the ^{1}H channel during contact time. Solid state ^{13}C spectra were referenced to an external adamantane peak at δ 38.3 (Varian) or to an external glycine methylene peak at δ 43.4 (Bruker) and were referenced to tetramethylsilane at δ 0.0. Proton spectra were obtained at 500 MHz on a Varian Inova-500 spectrometer at room temperature without spinning at Trinity University. Spectra were referenced in $CDCl_3$ to TMS at δ 0.0. Typical 1D parameters were as follows: spectral width 12,000 Hz, pulse width 60°, delay time 1.0 s, acquisition time 1.0 s, and scan number 4.

Sugar Exudation

Sugars in general are produced in several steps by photosynthesis from carbon dioxide and water in the atmosphere, catalyzed by light ($6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$). These hexoses are further transformed through biosynthetic pathways into the disaccharide sucrose. The photosynthetic products, collectively called photosynthates, provide raw materials for further biosynthesis, energy for metabolic reactions, and other physiological functions (Gan et al. 2019). Movement of the photosynthates about the plant body to provide the carbon and energy needs of the entire organism is called translocation. The photosynthate-donating parts of the plant are known as sources, and the photosynthate-receiving parts as sinks. Sucrose is the principal carbohydrate that is translocated in plants (Julius et al 2019, Stein and Granot 2019). Sucrose is translocated from sources to sinks via the phloem vascular tissue (Hennion et al. 2019). Sucrose also can form part of plant defense systems (Trouvelot et al. 2014, Formela-Luboińska et al. 2020).

Stress in plants, whether biotic (microbial or feeding by herbivores) or abiotic (drought, pruning, wind/ice damage, or defoliation), often leads to fluid exudation, which occurs through surface lesions that can lead directly to sucrose-containing tissues. Exudation also can occur in unstressful situations. Above-ground plant structures can secrete sucrose or other chemicals, possibly to reward

pollinators (Lin et al. 2014, Nepi et al. 2009, Roguz et al. 2018, Tiedge and Lohaus 2018). The mode of sucrose exudation is reminiscent of exudation of terpenoid resins, which are the materials from which copal and amber derive through long-term maturation (Lambert et al. 2008). Exudation has been stimulated, usually by cutting, and the molecular composition of the resulting liquid analyzed. Sucrose is an important component in many cases, all involving stimulated exudation from soft tissues such as leaves (Braun 2022, Chardon et al. 2022, Schnieder et al. 2022, Stallmann, Pons, and Schweiger 2022). To our knowledge, aside from sugar cane and maple, adventitious appearance of pure, solid sucrose on above-ground plant parts has not been observed previously.

Results and Discussion

Figure 2 illustrates the solid state ^{13}C NMR spectrum of sample 1462 of the exudate from *S. officinarum* from the Materia Medica Collection at the National Museum of American History, labeled "rock candy" and "possibly processed." The ^{13}C spectrum of sample 1463 from the same source was essentially the same. The strongest peak at δ 102 is from the anomeric carbon C2 of the five-membered fructose ring (see Figure 1 for the numbering system). The anomeric carbon C1 of the six-membered glucose ring is at δ 93. Both carbons are shifted to high frequencies because they are attached to two oxygen atoms. The resonance at δ 82 is from fructose C5. The peaks in the range δ 68-72 are from all the remaining CHOH tertiary carbons (fructose C3 and C4, glucose C2, C3, C4, and C5), and the broad peaks at δ ca. 60 are from the three CH_2OH secondary carbons (fructose C1 and C6, glucose C6). Literature comparisons were from the high-resolution spectrum recorded in D_2O, which shows minor differences from the results in the solid state (Fontana 2014, Kapaev 2014). The spectrum (Figure 3) of the unprocessed exudate sample 2118 from the sugar maple tree *A. saccharum* is sharper because of the use of newer instrumentation. Figure 4 shows the carbon spectrum of the exudate sample 1500 from wrinkled viburnum (sample 1500, *V. rhytidophyllum*), and Figure 5 the carbon spectrum of the exudate from Texas mountain laurel (sample 1592, *D. secundiflorum*). The viburnum and mountain laurel exudate spectra are very similar to those of sugar cane and sugar maple exudate. Figures 4 and 5 contain small carbonyl resonances at δ 176, which fall into the range for esters and lactones. They may derive from the open sugar form or from impurities (<3%)

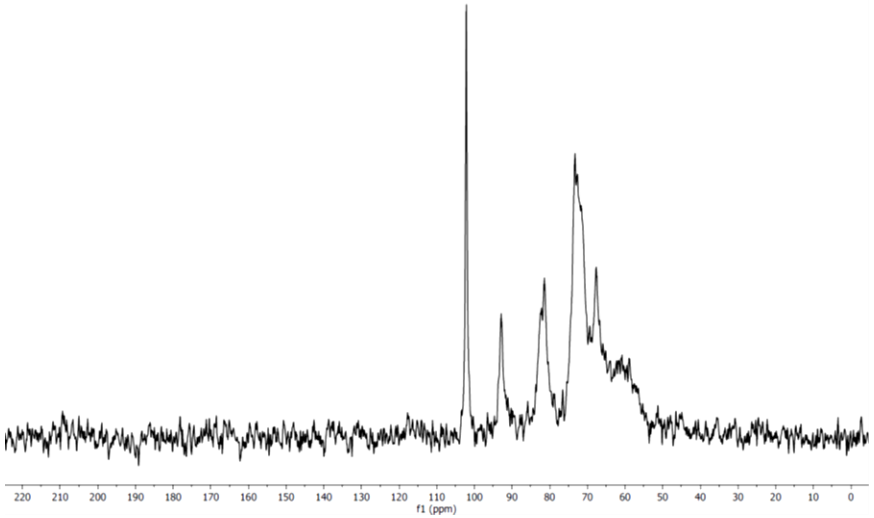

Figure 2. The solid state 100 MHz ^{13}C spectrum of sample 1462 *S. officinarum* with cross polarization and magic angle spinning.

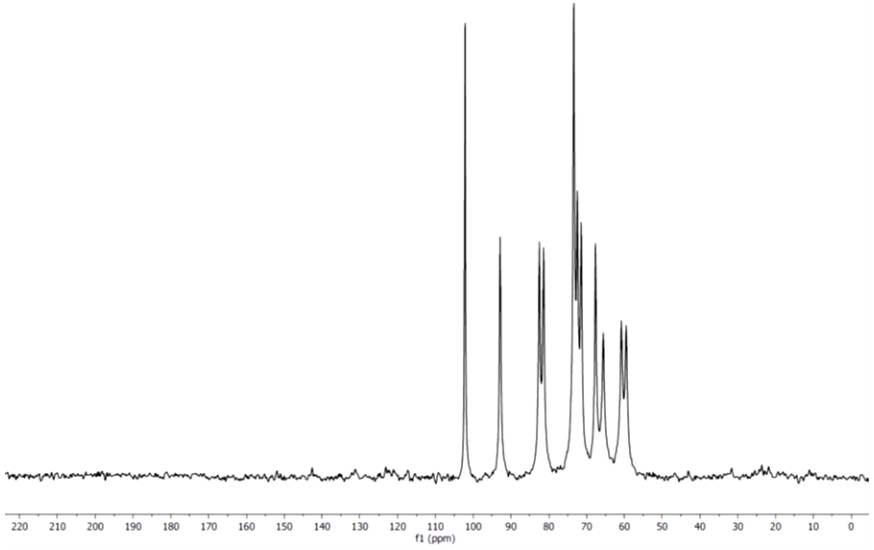

Figure 3. The solid state 100 MHz ^{13}C spectrum of sample 2118 *A. saccharum* with cross polarization and magic angle spinning.

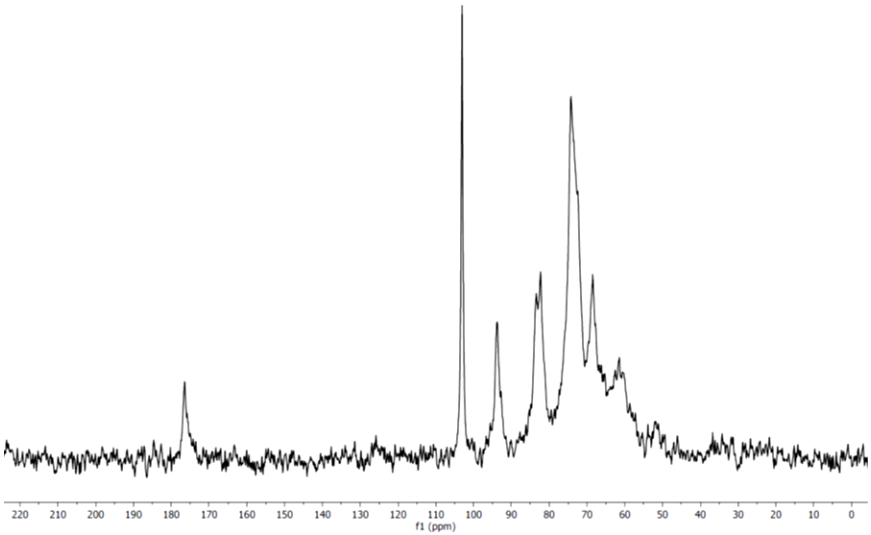

Figure 4. The solid state 100 MHz ^{13}C spectrum of sample 1500 of *V. rhytidophyllum* with cross polarization and magic angle spinning.

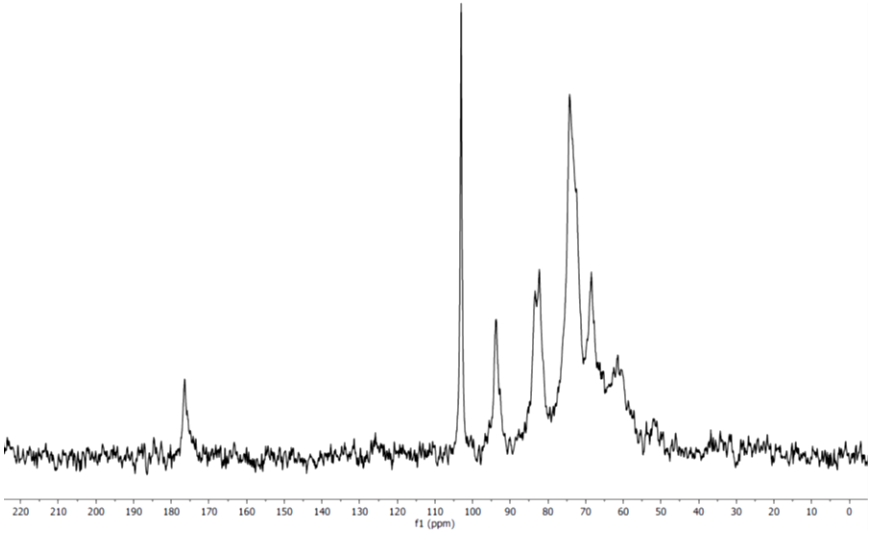

Figure 5. The solid state 100 MHz ^{13}C spectrum of sample 1592 of *D. secundiflorum* with cross polarization and magic angle spinning.

The sucrose samples were insoluble in $CDCl_3$ but gave excellent ^1H spectra in deuterated dimethylsulfoxide ($CD_3(SO)CD_3$, DMSO-d_6). Figure 6 presents the spectrum of sample 1192 of *S. officinarum* from sugar cane and probably

processed, and Figure 7 that of sample 2118 of *A. saccharum*. Both spectra replicate that of a sample of table sugar we recorded for comparison. Figure 8 is of sample 1500, *V. rhytidophyllum*, and Figure 9 of sample 1592 of *D. secundiflorum* in DMSO-d_6, again nearly identical although on slightly different scales. The ^{1}H spectra give a better idea of purity, which is >97% in all cases and probably >99% in Figures 6 and 7. The spectra in Figures 8 and 9 reveal small impurities, <3%.

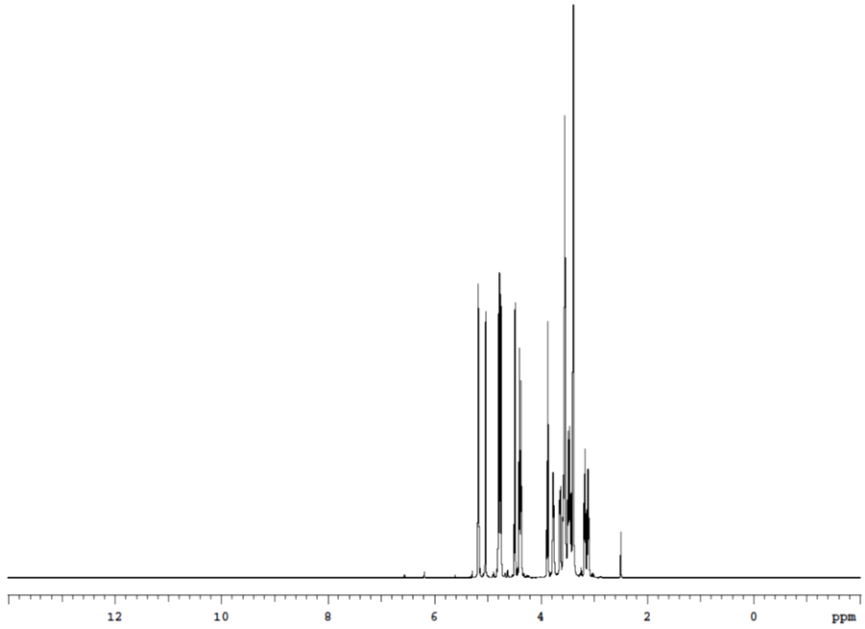

Figure 6. The 500 Hz ^{1}H spectrum in DMSO-d_6 of sample 1462 *S. officinarum*.

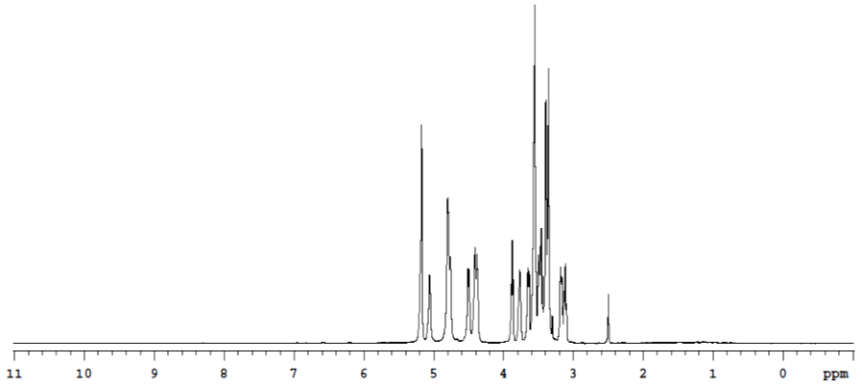

Figure 7. The 500 Hz ^{1}H spectrum in DMSO-d_6 of sample 2118 *A. saccharum*.

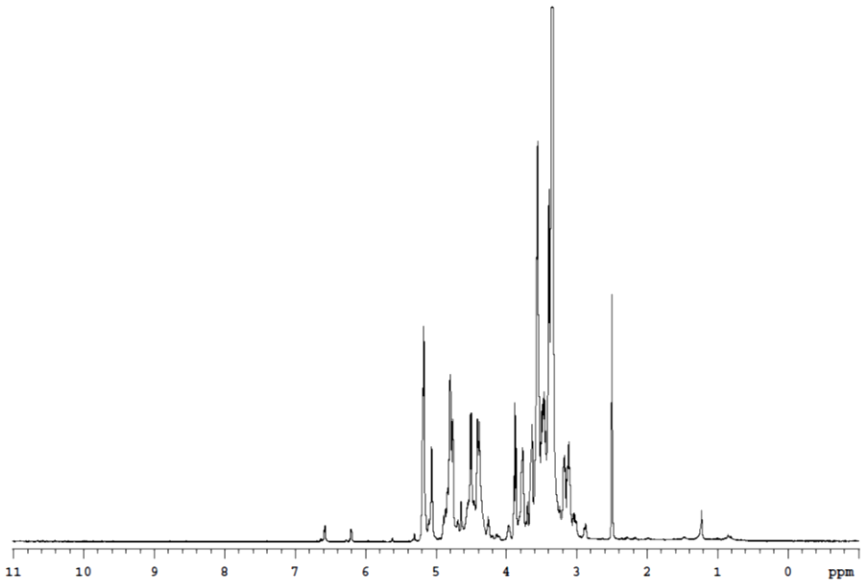

Figure 8. The 500 Hz ^{1}H spectrum in DMSO-d_6 of sample 1500 of *V. rhytidophyllum*.

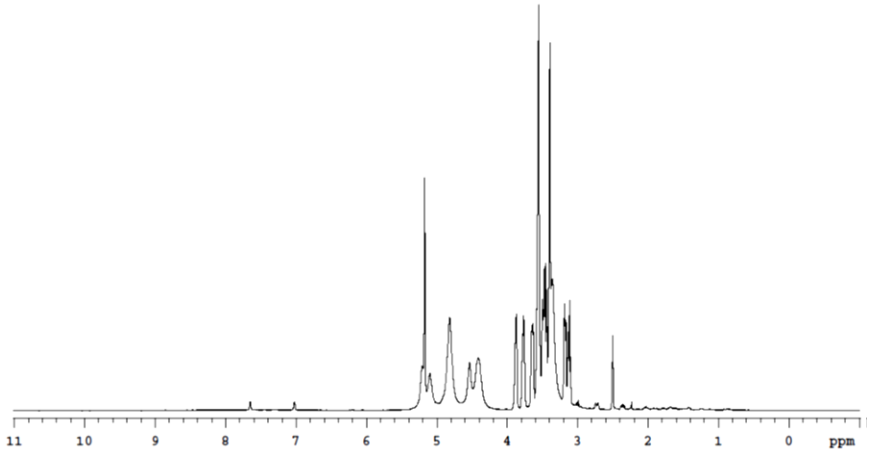

Figure 9. The 500 Hz ^{1}H spectrum in DMSO-d_6 of sample 1592 of *D. secundiflorum*.

Although the production of sucrose from cane sugar and maple sugar, respectively from *S. officinarum* and *A. saccharum*, normally involves extensive processing, sample 2118 was collected directly from the trunk of a sugar maple tree, was allowed to dry, and was subjected to ^{13}C NMR analysis without processing. The spectra of the samples from the wrinkled viburnum (Figure 4) and Texas mountain laurel (Figure 5) also were unprocessed and indicate that pure sucrose is produced as a plant exudate on the surface of a variety of plants. Whereas sugar cane is a grassy monocot, sugar maple and mountain laurel are eurosid trees, and wrinkled viburnum is a euasterid bush. These results indicate that pure sucrose occurs as an exudate in a variety of plants.

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