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Enumeration of hydroxyl groups of sugars and sugar alcohols by aqueous-based acetylation and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

Anthony Adeuya*,† and Neil P. J. Price

USDA-ARS-NCAUR, Renewable Product Technology Research Unit, 1815 North University Street, Peoria, IL 61604, USA

RATIONALE: The properties of carbohydrates are determined in part by the number and stereochemical arrangement of their hydroxyl groups. To facilitate their analysis, rapid methods are needed to identify and enumerate hydroxyl groups in sugars and polyalcohols, especially methods that are water-based.
METHODS: The present report details the results of an alternative method for identification and enumeration of hydroxyl groups in aqueous media. It employs vinyl acetate to selectively derivatize hydroxyl groups in analytes, followed by analysis of the reaction mixtures by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS).
RESULTS: The method has been applied to several single and multi-component mixtures of monosaccharides and polyalcohols. The O-acetylated products were analyzed without chromatographic separation or purification by MALDI-TOF-MS. The mass spectra revealed consecutive ion peaks that are separated by 42 mass units as a consequence of displacement of one hydroxyl hydrogen by one acetyl group.
CONCLUSIONS: A rapid and aqueous-based method is described to enumerate the hydroxyl groups in carbohydrates. The number of ion peaks due to derivatized products is determined by MALDI-TOF-MS, and corresponds to the number of free hydroxyl groups in the analyte. The method is applicable to both single and multi-component mixtures. Published 2011. This article is a US Government work and is in the public domain in the USA.

Carbohydrates and polyalcohols are of great importance in both the pharmaceutical and food industries. For example, polyalcohols are used as fillers in drugs and therefore are one of the by-products from drug metabolism. Carbohydrates and polyalcohols, like other hydroxyl group containing analytes, are typically characterized by gas chromatography (GC) and liquid chromatography (LC) based methods.[1–6] Analysis by GC usually requires tedious and time-consuming conversion of the hydrophilic polyalcohols into their lipophilic derivatives that are suitable for in-line pre-separation by GC.[3] Permethylation, peracetylation and trimethylsilylation are commonly used for this purpose.[7–11] Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has extended the usefulness of mass spectrometry in recent years. This technique, unlike electron impact (EI) ionization, generally gives molecular weight information as sample fragmentation is minimized with the matrix used for MALDI analysis.[12]

Studies have proposed hydroxyl group identification and enumerating methods that include ion-molecule derivatization reactions in a Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer and hydrogen/deuterium (H/D) exchange by MALDI-TOF-MS.[13,14] These methods require specialized instrumentation and a high degree of expertise. We recently reported a rapid and facile method of identifying and enumerating hydroxyl groups in sugars and polyalcohols by trimethylsilyl derivation reaction coupled with MALDI-TOF-MS.[15,16] The method can only be used in characterizing dry samples because of the inability to prepare trimethylsilyl derivatives in the presence of moisture.

The present report details the results of an alternative method for identification and enumeration of hydroxyl groups in aqueous media. This method employs a modified GC/MS per-*O*-acetylation method procedure where vinyl acetate selectively derivatized hydroxyl groups in analytes, followed by analysis of the reaction mixture by MALDI-TOF-MS. MALDI-TOF mass spectra of acetyl-derivatized analytes revealed consecutive ion peaks that are separated by 42 Da. This 42 mass unit is due to a net substitution of one hydroxyl hydrogen atom by one acetyl group. The number of peaks due to derivatized products corresponds to the number of free hydroxyl groups in the analyte. This method was applied to several single and multi-component mixtures of monosaccharides and polyalcohols. In addition, like the results of previous studies,[15,16] more information is obtained for each sugar or polyalcohol due to the presence of the reactant (starting sugar or polyalcohol), partial and fully derivatized product ions in

* *Correspondence to:* A. Adeuya, USDA-ARS-NCAUR, Renewable Product Technology Research Unit, 1815 North University Street, Peoria, IL 61604, USA.
E-mail: anthony.adeuya@fda.hhs.gov

† Current address: 3900 NCTR Road, Jefferson, AR 72079, USA.

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the mass spectrum. This can be very valuable when multi-component mixtures are being analyzed. This method can be used in analysis of hydroxyl groups containing compounds in food, drugs and environmental matrixes.

EXPERIMENTAL

Chemicals

All monosaccharides used were from the collection at NCAUR. Erythritol, xylitol, vinyl acetate and the matrix reagent (2,5-dihydroxybenzoic acid and acetonitrile) were obtained from Sigma Aldrich (St. Louis, MO USA). Mannitol was obtained from Fisher Scientific (Fair Lawn, NJ, USA). All samples were used as received.

Derivatization reactions

Recently, Shi and coworkers reported the results of a study that showed excellent total derivatization of hydroxyl groups.[7] In this study, the reported method has been modified to obtain all the partial and fully derivatizated products of hydroxyl group containing analytes. In brief, about 2 mg of sample were dissolved in a vial containing 20 μL of vinyl acetate and 100 μL of 3% Na_3PO_4 buffer solution at room temperature (pH of approximately 12). (For the mechanism of the reaction, see Scheme 1.) The mixture was vortexed, allowed to stand for about 3 to 5 min and analyzed by MALDI-TOF-MS without purification or extraction. For the multi-component study, about 1 mg of each component was dissolved in 30 μL of vinyl acetate with 150 μL of the buffer solution. Special care must be taken when working with vinyl acetate. Its vapor causes eye and respiratory irritations, and it is a fire hazard according to the United States Occupational Safety and Health Administration (OSHA).[17]

MALDI-TOF-MS

Acetylation reaction mixtures (about 0.5 μL) were spotted on the MALDI-TOF MS stainless steel target followed by 2 μL of a saturated matrix solution (2,5-dihydroxybenzoic acid, DHB, in acetonitrile). The resulting cocrystal was allowed to dry at room temperature and the target was introduced into the mass spectrometer via the probe carrier. Measurements were acquired in the positive ion reflectron mode on an Omniflex time-of-flight mass spectrometer (Bruker Daltonics Inc., Billerica, MA, USA). A 200 ns pulsed ion extraction was used with ion sources 1 and 2 set to 19.00 kV and 13.6 kV, respectively. The reflectron voltage was set to 20 kV. Ions were excited at 337 nm, typically at 65% of 150 μJ maximum laser power output. Measured mass spectra were an average of 200 laser shots. The instrument was calibrated externally by a series of malto-oligosaccharides.

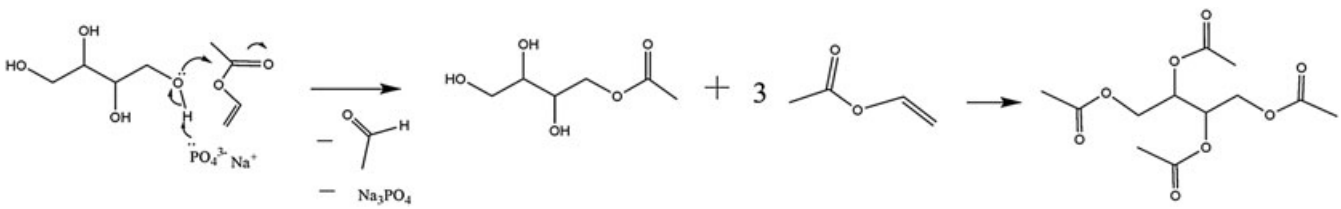
RESULTS AND DISCUSSION

MALDI-TOF-MS coupled with aqueous-based acetylation has been used successfully in determining the number of free hydroxyl groups in monosaccharides (glucose, ribose, galactose) and polyalcohols (erythritol, mannitol, and xylitol). The product mixture of each analyte derivative was detected by using MALDI-TOF-MS in the positive ion mode as sodium adducts represented as $[M + Na]^+$ and $[M–xH + xAc + Na]^+$ (where x is the number of hydroxyl or Acetyl (Ac) groups) for the starting material and the acetyl derivatized product, respectively. The presence of multiple hydroxyl groups in an analyte results in consecutive derivatized species that are separated by 42 Da in molecular mass, corresponding to the mass increase as the result of displacement of one hydrogen atom by one acetyl group.

Erythritol was derivatized with vinyl acetate as described in the Experimental section and analyzed by MALDI-TOF-MS. A total of five peaks, separated by 42 mass units, were observed in the mass spectrum of the analyzed reaction mixture (Fig. 1(a)). These peaks are due to the starting material (erythritol), three partially derivatized erythritols and the fully acetyl derivatized erythritol at *m/z* 145, 187, 229, 271, and 313, respectively.

Similarly, with xylitol the number of observed peaks due to differently derivatized products corresponds to the number of free hydroxyl groups in xylitol. There are five free hydroxyl groups on xylitol and a total of six mass spectra peaks were observed at *m/z* 175, 217, 259, 301, 343, and 385 due to the starting material, xylitol, and the first through fifth derivatized products, respectively. The peak at *m/z* 535 corresponds to the sodium adduct of the fully acetylated species $[xylitol–5\ H + 5Ac + Na]^+$ (Fig. 1(b)).

The reactivity of mannitol towards vinyl acetates is similar to that of erythritol and xylitol. As observed previously, the number of observed peaks due to differently derivatized products correlates with the number of free hydroxyl groups in mannitol. Hence, mannitol has six free hydroxyl groups and a total of seven mass spectra peaks were observed at *m/z* 205, 247, 289, 331, 373, 415, and 457 due to the starting material, mannitol, and the first through sixth derivatized products, respectively. It is important to note that the MALDI-TOF mass spectra revealed the fully derivatized product peaks for both xylitol and mannitol (Fig. 1(c)) unlike those obtained in a previous FT-ICR-MS study.[13] In addition, there are no fragment ions due to ethane losses and the results are therefore easier to interpret.



Scheme 1. Proposed mechanism for the reaction of erythritol and vinyl acetate.

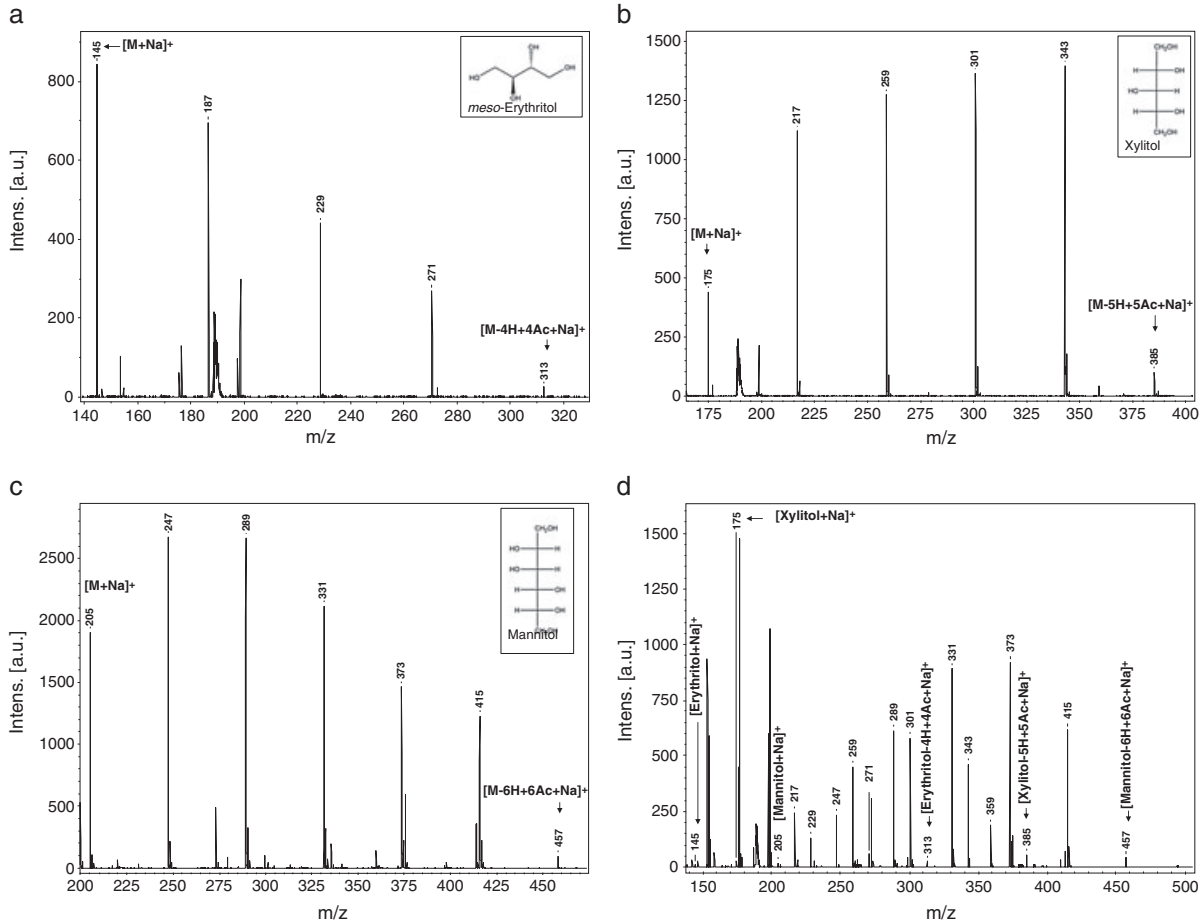


Figure 1. MALDI-TOF mass spectra of the reaction products of erythritol (a), xylitol (b), mannitol (c), and erythritol/mannitol/xylitol mixture (d) with vinyl acetate. The peak denoted as $[M+Na]^+$ represents the ion for the starting polyalcohol (*m/z* 145, 175, 205 for erythritol, xylitol and mannitol, respectively). The other peaks are due to sodium adducts of the acetyl derivatives. Unlabeled peaks are due to matrix and impurity ions.

The hydroxyl group enumeration method described above can be applied to multi-component mixtures. This was shown by treating a mixture containing erythritol, xylitol and mannitol with vinyl acetate and analyzing the resulting mixture by MALDI-TOF-MS. The measured mass spectrum (Fig. 1(d)) reveals the same information as the measured mass spectra for single components (Figs. 1(a), 1(b) and 1(c)). Hence, a total of 18 peaks are evident for the mixture, 5, 6 and 7 for erythritol, xylitol and mannitol, respectively.

The results obtained for the three sugars examined (ribose, fucose and glucose and a mixture containing all three) are shown in Fig. 2. Like the results from the polyalcohols study, it revealed peaks that are due to starting material, the partially derivatized and fully derivatized products for each sugar. Hence, five characteristic ions are observed for the 6-deoxysugar, fucose (*m/z* 187, 229, 271, 313 and 355); five for ribofuranose (*m/z* 173, 215, 257, 299 and 341); and six for glucopyranose (*m/z* 203, 245, 287, 329, 371 and 413). Noticeably, the cyclic hemiacetal forms of these sugars are retained during the reaction procedure, resulting in the corresponding five- or six-membered cyclic O-acetylated derivatives.

Like the result of a previous study,[15] repeated acetylation and MALDI-TOF-MS measurements for the single and multi-component analyte mixtures showed varied peak intensities. Qualitatively, the same numbers of peaks were obtained for corresponding trials. This observed variation may be due to the varying ionization efficiency and a well-known phenomenon of non-uniform co-crystalization of analyte/matrix mixture on the MALDI target plate.[18]

CONCLUSIONS

MALDI-TOF-MS coupled with acetyl derivatization described in this work may be used to identify and enumerate the hydroxyl groups in monosaccharides and polyalcohols in aqueous media. This approach is rapid, requires little or no expertise in organic synthesis, and there is no need for sample purification prior to mass spectrometry measurements.

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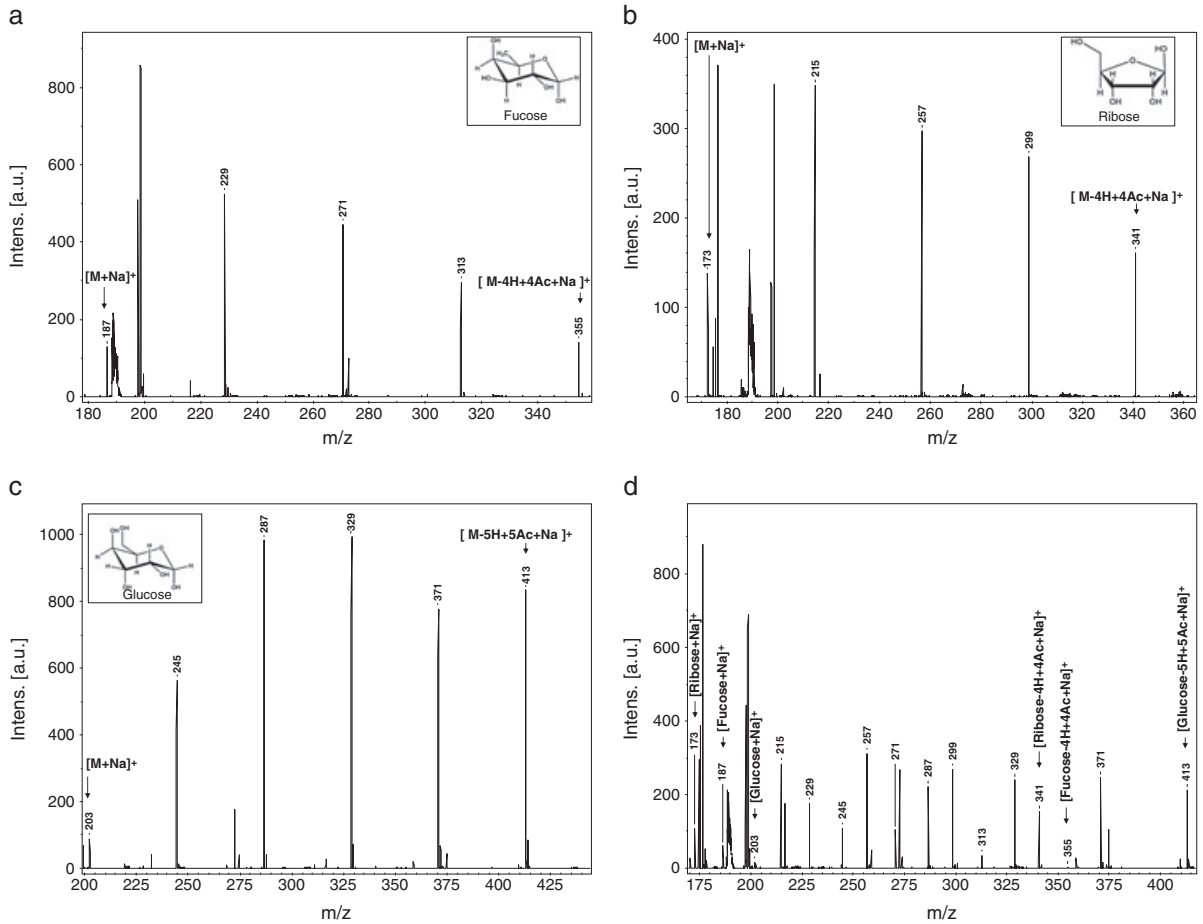

Figure 2. MALDI-TOF mass spectra of the reaction products of fucose (a), ribose (b), glucose (c), and glucose/fucose/ribose mixture (d) with vinyl acetate. The peak denoted as $[M+Na]^+$ represents the ion due to a non-derivatized or the starting monosaccharide (*m/z* 187, 173, 203 for fucose, ribose and glucose, respectively). The other labeled peaks are due to sodium adducts of the acetyl derivatized products. Unlabeled peaks are due to matrix and/or impurity ions.

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