Journal of Chromatography A, 1372 (2014) 221–227

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Characterization of trimethylsilyl ethers of iminosugars by gas chromatography–mass spectrometry

S. Rodríguez-Sánchez, A.I. Ruiz-Matute, M.L. Sanz, A.C. Soria *

Instituto de Química Orgánica General (CSIC), Juan de la Cierva, 3, 28006 Madrid, Spain

ARTICLE INFO

Article history:
Received 2 August 2014
Received in revised form 11 October 2014
Accepted 25 October 2014
Available online 1 November 2014

Keywords:
Iminosugars
Gas chromatography
Mass spectrometry
Trimethylsilylation
Linear retention indices
Aglaonema treubii

ABSTRACT

A combination of gas chromatographic retention data (linear retention indices) on two different stationary phases (100% methyl- and 50% phenylmethylsilicone) and electron impact mass spectrometric relative abundances for characteristic *m/z* ratios of 12 trimethylsilylated piperidine and pyrrolidine iminosugars are reported. These results have been related to their structural features and applied to the characterization of iminosugar composition of an *Aglaonema treubii* root extract. Seven iminosugars were detected in this extract, two of them were described for the first time.

1. Introduction

Iminosugars are polyhydroxyalkaloids consisting of monocyclic or bicyclic derivatives of pyrrolidine, piperidine, pyrrolizidine, indolizidine or nortropane ring structures [1]. Many of them are able to inhibit glycosidases and to interfere with sugar receptors [1]. They can be found in natural sources such as plants from the Araceae, Leguminosae, Moraceae, Hyacinthaceae, Campanulaceae, etc. families and microorganisms such as *Streptomyces*, *Bacillus*, etc. [2], but they can also be synthetically produced [3–5].

Simple mixtures or individual iminosugars are commonly analyzed by high performance liquid chromatography (HPLC) [6–8]. However, the analysis of complex mixtures such as those extracted from natural sources is not straightforward considering the low abundance of iminosugars and the often poor separation among the different iminosugars and among these bioactives and other coextracted low molecular weight carbohydrates (LMWC). Gas chromatography (GC) provides good resolution and sensitivity, although a previous derivatization step is mandatory [9,10]. Trimethylsilylation confers iminosugars and other LMWC the necessary stability and volatility for their GC analysis [11]. Although special emphasis has been paid to the optimization of silylation reagents [1,9] and chromatographic conditions [10], there is still a lack of comprehensive studies regarding the different retention of iminosugars depending on the stationary phase. In this sense, Nash et al. [12] reported the separation of seven trimethylsilyl (TMS) derivatives of naturally occurring polyhydroxyalkaloids ((2R,3S)-2-hydroxymethyl-3-hydroxypyrrolidine, 1,4-dideoxy-1,4-imino-D-arabinitol (DAB-1), fagomine, 2,5-dideoxy-2,5-imino-D-mannitol (DMDP), swainsonine, 1-deoxynojirimycin (DNJ) and castanospermine) in two different GC columns (methyl and phenylmethylsilicone). However, retention indices of these compounds were not provided and chromatographic data on a larger number of naturally occurring iminosugars would be useful for their further identification in real samples.

Additional qualitative information, valuable for the characterization of unidentified iminosugars, can be obtained by coupling gas chromatography to mass spectrometry (GC–MS). Characteristic electron impact (EI)MS ions such as those originated by the loss of methyl and/or -CH_2OTMS groups have been provided for some trimethylsilylated iminosugars (e.g. DMDP and α-homonojirimycin (α-HNJ) [13]; australine [14]). Moreover, EI mass spectra of DAB-1, DNJ and swainsonine [15] and of several calystegines [16–19] have also been reported.

Although a combination of GC retention data and mass spectral patterns (abundance of characteristic *m/z* ratios) could provide useful information for the characterization/identification of iminosugars, these data are only available for a very few compounds (e.g.: linear retention indices (I^T) and (EI)MS fragmentation of fagomine and DNJ [9]). In the present work, I^T on two

* Corresponding author. Tel.: +34 912587559; fax: +34 915644853.
E-mail address: acsoria@iqog.csic.es (A.C. Soria).

http://dx.doi.org/10.1016/j.chroma.2014.10.084
0021-9673/

stationary phases and (EI)MS data of 12 trimethylsilylated piperidine and pyrrolidine iminosugars are provided. These data have been correlated with their structures in an attempt to help in the further identification of these compounds in natural sources such as *Aglaonema treubii* root extracts.

2. Materials and methods

2.1. Standards and samples

Analytical standards of DNJ, 1-deoxymannojirimycin (DMJ), *N*-methyl-1-deoxynojirimycin (*N*-methyl-DNJ) and *N*-nonyl-1-deoxynojirimycin (*N*-nonyl-DNJ) were obtained from Sigma Chemical Co. (St. Louis, US). Deoxy-L-idonojirimycin (DIJ) and 1-deoxygalactonojirimycin (DGJ) were purchased from Toronto Research Chemicals Inc. (Ontario, Canada) and α-homonojirimycin (α-HNJ), *N*-methyl-*trans*-4-hydroxy-L-proline, 1-deoxyfuconojirimycin (DFJ) and DMDP were purchased from Dextra Laboratories (Reading, UK). Purity of all these standards was higher than 95%. Miglitol (1-(2-hydroxyethyl)-2-(hydroxymethyl)-piperidine-3,4,5-triol) was acquired in a local pharmacy and fagomine (1,5-imino-1,2,5-trideoxy-D-arabino-hexitol) was extracted from mulberry leaves, as indicated by Rodríguez-Sánchez et al. [9]. Solutions (1 mg mL^{-1}) of standards in ultrapure Milli-Q (Millipore) water were prepared for analysis.

A plant of *A. treubii* was acquired in a local garden center in Madrid. LMWC were extracted from *Aglaonema* roots using a Pressurized Liquid Extraction system as previously described by Rodríguez-Sánchez et al. [20]. In brief, 0.08 g of roots were extracted with 0.8 mL of ultrapure water at 10 MPa and 50 °C for 5 min.

2.2. Derivatization

One mL of standard solutions or *A. treubii* root extract was mixed with 0.5 mL of internal standard (phenyl-β-D-glucoside 1 mg mL^{-1}) and evaporated under vacuum prior to carbohydrate derivatization. A two-step derivatization procedure (oximation + silylation) [21] was carried out prior to GC–MS analysis of iminosugars. Previous studies have shown that this methodology results in a better GC resolution of iminosugars and other bioactive carbohydrates from other coextractives usually taking part of the complex mixtures present in plant extracts [9].

Oximes were obtained by addition of 350 μL of a solution 2.5% hydroxylamine chloride in pyridine after 30 min at 75 °C. They were then silylated with hexamethyldisilazane (350 μL) and trifluoroacetic acid (35 μL) at 45 °C for 30 min. Under these derivatization conditions only one peak is observed for each iminosugar, corresponding to the *O*-persilylated form, and *N*-silylated compounds are not obtained, as previously indicated by Rodríguez-Sánchez et al. [9]. After reaction, samples were centrifuged at 4400 *g* for 10 min, and 1 μL of supernatant was injected into the GC injection port.

2.3. GC–MS analysis

GC–MS analyses (n = 3 replicates) were carried out following the validated method of Rodríguez-Sánchez et al. [10], using a 7890A gas chromatograph coupled to a 5975C quadrupole mass detector (Agilent Technologies, Palo Alto, CA, USA). Analyses were carried out on a 30 m × 0.25 mm *i.d.*, 0.25 μm d_f, methylpolysiloxane capillary column (HP-1, Agilent Technologies, Palo Alto, CA, USA) and a 50% phenylmethylpolysiloxane column (BPX-50, SGE Europe Ltd, UK) of the same dimensions, using helium at ~1 mL min^{-1} as carrier gas. For both columns, the oven temperature was programmed from 100 °C to 200 °C (15 min) at a heating rate of 15 °C min^{-1} and finally programmed to 300 °C (10 min) at 15 °C min^{-1} (total analysis time of 38 min). Injections were carried out in split mode (1:20) at 240 °C. The transfer line and ionization source were thermostated at 280 and 230 °C, respectively. Mass spectra were recorded in EI mode at 70 eV within the mass range *m/z* 35–650. Acquisition was done using HPChem Station software (Agilent Technologies, Palo Alto, CA, USA). I^T were calculated from retention times of TMS iminosugars and suitable *n*-alkanes, as described in [22,23].

3. Results and discussion

Table 1 shows I^T data on two stationary phases (methyl- and phenylmethyl silicone) and abundances of characteristic ions in the EI mass spectra of the 12 *O*-persilylated iminosugars under study. Full scan mass spectra for these compounds can be found in Fig. 1S of Supplementary Material.

3.1. Chromatographic data

No change in the elution order of iminosugars on both stationary phases (HP-1 and BPX-50) was observed (Table 1). As reported by Nash et al. [12], a close correlation of retention time with degree of hydroxylation was found (see Fig. 2S of Supplementary Material). Thus, *N*-methyl-*trans*-4-hydroxy-L-proline was the first compound to elute, followed by those iminosugars with three, four, etc. —OTMS groups. As expected, among the compounds with identical molecular formula (e.g. DFJ and fagomine), the retention was affected by the nature of the substituents and their corresponding slight differences in polarity.

Regarding iminosugars with four —OTMS groups, the lowest linear retention index was observed for DMDP followed by DIJ, DMJ, DGJ and DNJ. The potential higher volatility of DMDP, a five-membered ring with two —CH_2OTMS substituents, could justify its lower retention. It is also worth noting the differences in I^T values for iminosugars only differing in the orientation (axial or equatorial) of their hydroxyl groups (e.g. DIJ, DMJ, DGJ and DNJ). This behavior is similar to that found for trimethylsilylated monosaccharides [24]. In agreement with this, Asano et al. [13] reported differences in chromatographic retention for various iminosugar isomers and, as an example, they found that α-homomannojirimycin (α-HMJ) eluted before α-HNJ using a BPX-5 capillary column.

In both columns, *N*-methyl-DNJ (with molecular formula $C_7H_{15}NO_5$) eluted before DNJ (molecular formula $C_6H_{13}NO_4$). Higher retention indices were observed for α-HNJ, miglitol and *N*-nonyl-DNJ.

3.2. Mass spectra

As expected for persilylated sugars, molecular ions were not observed in the spectrum of any of the standards under study; $[M-15]^+$ ions were, however, present in all of them (see Table 1 and Fig. 1S of Supplementary Material).

TMS *N*-methyl-*trans*-4-hydroxy-L-proline mass spectrum showed a characteristic *m/z* 172 ion ($[M-117]^+$, base peak) corresponding to the loss of -COOTMS from the molecular ion. This fragment loses a TMSOH group giving an abundant ion at *m/z* 82. Kite and Hughes [25] reported a similar fragmentation pattern for hydroxypipecolic acids. In these compounds, the intensity of the ratio between *m/z* 56 and *m/z* 82 ions was found to be crucial to distinguish between 4- and 5-hydroxypipecolic acids; however, the former ion was not present in the mass spectrum of *N*-methyl-*trans*-4-hydroxy-L-proline, where the loss of a methyl group from the molecular ion (*m/z* 274 ion) was detected at relatively high intensity.

Noticeable differences in the MS fragmentation pattern of TMS derivatives of fagomine and DFJ were found. For fagomine,