Short communication

Synthesis of L-xylo-hexos-2-ulose (L-sorbosone) and its characterisation by chromatographic and spectroscopic techniques

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Abstract

A modified pathway to synthesize on large scale L-xylo-hexos-2-ulose (L-sorbosone), a potential intermediate in the biosynthesis of L-ascorbic acid in some plants, is described. The determination of the purity by thin-layer chromatography, high-performance liquid chromatography, capillary gas chromatography (cGC) and cGC–mass spectrometry (MS) is discussed. The product is for the first time unambiguously characterized by ^{13}C NMR and electrospray MS. © 1998 Elsevier Science B.V. All rights reserved.

1. Introduction

It has been postulated that L-sorbosone is a potential intermediate in the biosynthesis of L-ascorbic acid in some plants [1,2]. In order to gain better insight into this biosynthetic pathway, it is necessary to have L-sorbosone in very pure state and, if possible, in high quantities. The conversion of L-sorbose to L-sorbosone by means of enzymatic oxidation with L-sorbose dehydrogenase has been described [3,4]. This enzyme, however, is not commercially available and must be isolated from microorganisms and then immobilized [4]. An alternative can be found in the immobilization of cells con-taining the enzyme [3]. These methods are rather laborious and definitely unsuitable for production of L-sorbosone on a multigram scale. To the best of our knowledge, only one synthetic approach has been described in the patent literature [5]. We were, however, unable to synthesize L-sorbosone in sufficiently high purity and quantity applying this method. Moreover, until now, no data on chromatographic or spectroscopic characterization for unambiguous structure elucidation and purity check have been published. We therefore developed a different synthetic pathway and evaluated thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), capillary gas chromatography (cGC) and cGC–mass spectrometry (MS) to determine the purity of the synthesized product. The structure of L-sorbosone was confirmed by ^{13}C NMR

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spectroscopy and by electrospray (ES) MS of the product as such and of several derivatives.

2. Experimental

2.1. Chemicals and reagents

All solvents, chemicals and reagents were purchased from Aldrich or Acros. L-Sorbose was 98% from Aldrich. For the Swern reaction, dimethylsulfoxide and triethylamine were dried over calciumhydride while dichloromethane was dried over phosphorus pentoxide. Dowex 50-X8-100 was acidified with hydrochloric acid (10%) and washed with water until neutral pH.

2.2. Derivatization

Oximation was performed by placing 10 mg of the sample in a test tube with a PTFE stopper and adding 500 µl of a solution of 2.5 g hydroxylamine or methoxylamine hydrochloride in 100 ml pyridine. After vigorous shaking, the tube was placed in an oven at 80°C for 10 min. Excess reagent was removed under a stream of nitrogen and 500 µl pyridine was added. For further silylation 450 µl hexamethyldisilazane and 50 µl trifluoroacetic acid were added. The tube was closed and placed in an oven at 80°C for 10 min. The tube was immediately opened to remove ammonia and after cooling the derivatives were extracted with isooctane.

2.3. Synthesis

2.3.1. 2,3:4,6-Di-O-isopropylidene-α-L-sorbose
L-Sorbose (I) was reacted with acetone and sulfuric acid at room temperature according to a well known procedure [6] affording the bis-acetonide (II) (Fig. 1).

2.3.2. 2,3:4,6-Di-O-isopropylidene-α-L-sorbosone
The primary alcohol function of the protected sorbose was oxidized to the aldehyde (III) under mild Swern conditions [7] (Fig. 1). In a typical run, 58 mmol oxalyl chloride was dissolved in 90 ml dichloromethane under argon atmosphere. The solution was cooled to −60°C and 118 mmol di-

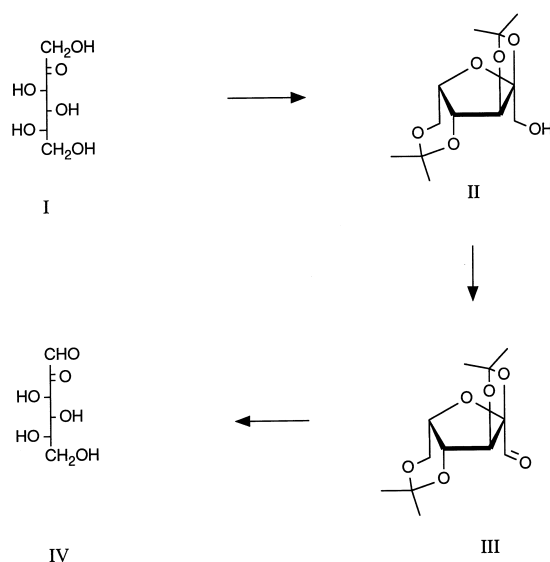
Fig. 1. Synthetic route for α-L-sorbosone.

methylsulfoxide dissolved in 90 ml dichloromethane was added over a period of 10 min. The solution was stirred magnetically for 5 min and 19 mmol (5 g) 2,3:4,6-di-O-isopropylidene-α-L-sorbose, dissolved in 45 ml dichloromethane, was slowly added over a period of 5 min while keeping the temperature at −60°C. A precipitate was formed. The suspension was stirred for 75 min at −60°C. The reaction was monitored by TLC [pentane–ethyl acetate (7:3), R_F alcohol=0.19; R_F aldehyde=0.08]. 251 mmol triethylamine was added over 15 min and a voluminous precipitate was formed. The temperature was slowly raised to room temperature overnight. A saturated sodium chloride solution was added and the layers were separated. The water phase was extracted six times with dichloromethane. The collected organic layers were washed consecutively with 5% phosphoric acid, saturated sodium hydrogencarbonate and brine, and dried over anhydrous magnesium sulfate. After filtration and evaporation of the solvent, the crude aldehyde was purified by column chromatography over silica gel (125 g silica 60, Merck). Elution was performed with pentane–ethyl acetate (6:4). A pale yellow oil which solidified upon standing was obtained in 72% yield. The following ^{1}H NMR data were obtained (500 MHz; δ in ppm; C[2]HCl$_3$; δ=9.68, s, 1H; δ=4.55, m, 1H; δ=4.36, m,

1H; δ=4.23, m, 1H; δ=4.12, m, 2H; δ=1.54, s, 3H; δ=1.44, s, 3H; δ=1.39, s, 3H; δ=1.34, s, 3H). Because the aldehyde is rather unstable, it was directly converted in L-sorbosone.

2.3.3. L-Sorbosone

The bis-acetonide was hydrolyzed using an acidic ion-exchange resin, thus simplifying the work-up and isolation of the crude L-sorbosone (IV) (Fig. 1). In a typical run, 1.166 g of 2.3:4.6-di-*O*-isopropylidene-α-L-sorbosone was dissolved in 11 ml tetrahydrofuran and 22 ml water and 5.84 g Dowex (50-X8-100-H$^+$) were added. The suspension was stirred for 24 h at 35–40°C. The reaction was monitored by TLC (dichloromethane–methanol–water, 7:3:0.4; R_F bis-acetonide=0.99; R_F L-sorbosone=0.24 and 0.07). The ion-exchange resin was filtered off, tetrahydrofuran was evaporated under reduced pressure and the residual aqueous solution was finally lyophilized. A pale yellow oil was obtained. This crude material was purified by column chromatography over silica gel (35 g silica 60, Merck). Elution was effected with dichloromethane–methanol–water (7:3:0.4). L-Sorbosone was obtained as a pale yellow amorphous powder in 89% yield.

2.4. Chromatographic techniques

2.4.1. Thin-layer chromatography

Precoated silica gel plates 60 GF$_{254}$, thickness 0.25 mm from Merck were used. The spots were visualized by spraying with a solution of phosphomolybdic acid (2.5%), cerium(IV) sulfate (1%) and sulfuric acid (6%) in water, followed by heating.

2.4.2. High-performance liquid chromatography

A Kontron Instruments 422 LC equipped with refractive index (RI) detector was used. Samples dissolved in the eluent at 1% were injected in a Rheodyne valve of 10 μl onto a 30 cm×3.9 mm I.D., 10 μm Carbohydrate column (part No. 84038, Waters). The mobile phase was acetonitrile–water (6:4) containing 3 m*M* potassium phosphate buffer (pH 6.0). The flow-rate was set at 1.0 ml/min and analyses were performed at room temperature.

2.4.3. Capillary gas chromatography–mass spectroscopy

Analyses were performed on a HP 5890 GC coupled to a HP 5972 mass-selective detector (Hewlett-Packard). Two fused-silica open tubular capillary columns of 30 m×0.25 mm I.D. were used: (a) coated with 0.25 μm diphenyl(5%)dimethylsilicone (HP5MS) and (b) coated with 0.25 μm diphenyl(50%)dimethylsilicone (HP50+). Helium was used as carrier gas at 0.6 bar inlet pressure. The column was programmed from 60°C (2 min isotherm) to 250°C at 10°C/min.

A 1-μl volume was injected in the split mode (1/50) at 250°C.

2.5. Spectroscopic methods

2.5.1. Nuclear magnetic resonance

^{1}H NMR spectra were recorded on a 500 MHz (Brucker AN-500) and ^{13}C NMR spectra on a 50.3 MHz (Varian Gemini-200) in [2]H$_2$O. Chemical shifts are expressed in ppm versus tetramethylsilane, acetonitrile was added as internal standard.

2.5.2. Electrospray mass spectroscopy

ES-MS was performed on a Hewlett-Packard 5989B quadrupole instrument equipped with a high energy dynode detector and an atmospheric pressure ionization source with high-flow nebulizer. Nitrogen was used as nebulizing (80 p.s.i.) and drying (30 l/min) gas in the positive ion mode, while air was used under the same conditions for negative ion mode operation (1 p.s.i.=6894.76 Pa). Samples were introduced (10 μl) by means of flow injection analysis via a syringe pumping system (Harvard Apparatus 22) operated at a flow-rate of 20 μl/min with methanol–water (1:1) containing 5 m*M* ammonium acetate for the positive ion mode and with methanol only for the negative ion mode. Collision induced dissociation (CID) was performed at +50 and +200 V for positive ion operation and at −50 V for negative ion operation.

3. Results and discussion

During hydrolysis of 2,3:4,6-di-*O*-isopropylidene-α-L-sorbosone into α-L-sorbosone two spots ap-

peared in TLC namely at R_F 0.24 and 0.07. Moreover, a two-dimensional TLC run with the same eluent revealed that both products were interconverted. This observation may be explained by dimerization or hydrate formation during the separation which seems to be catalyzed by silica gel, although this is only speculative. HPLC on octadecyl silica C_{18} gave no satisfactory results either. Two broad peaks, partially overlapping, were observed. The two solutes were collected and re-injected but the same chromatogram was obtained. After collecting homogenous fractions and storing them at room temperature for several days, reinjection gives for all of them again the same broadened type of chromatogram. The sample and collected fractions were submitted to ^{13}C DEPT NMR and the spectra revealed the presence of a single compound only; the resonance frequencies of which are in complete agreement with the structure of α-L-sorbosone; δ (ppm)=97.36 CO, 89.32 CHO, 74.33, 71.20 and 69.78 CHOH and 62.13 ppm CH_2OH (Fig. 2). On the other hand, no conclusions could be drawn from ^{1}H NMR experiments because all resonances collapsed. Until now, no spectral information on α-L-sorbosone has been published for comparison, and further evidence was searched for by applying ES-MS. The negative ion spectrum with collision induced dissociation at −50 V shows the presence of m/z 177 $[M-H]^-$ which corresponds to the molecular ion and of m/z 161 $[M-OH]^-$. Upon oximation with hydroxylamine HCl, the molecular ion shifts to m/z 209 $[M+H]^+$ and with methoxylamine HCl to m/z 237 $[M+H]^+$. Both values are for ES-MS in the positive ion mode and a CID of +50 V. Silylation of the hydroximated derivative gave in the positive mode with CID +50 V, ions m/z 641 $[M+H]^+$, m/z 569 $[M+H-HOSi(CH_3)_3]^+$ and m/z 497 $[M+H-2HOSi(CH_3)_3]^+$. The ES-MS spectrum recorded in the positive ion mode and at +50 V CID for the methoximated and silylated α-L-sorbosone is shown
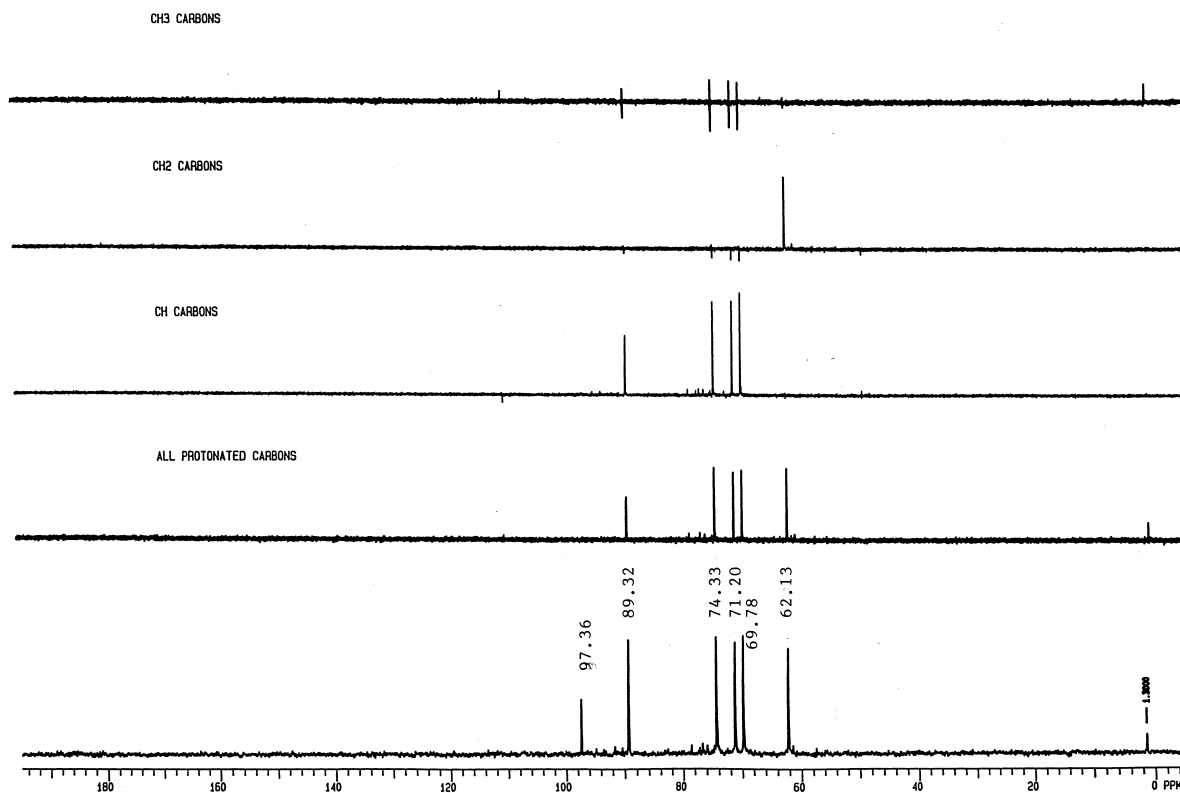

Fig. 2. ^{13}C NMR spectrum of α-L-sorbosone.
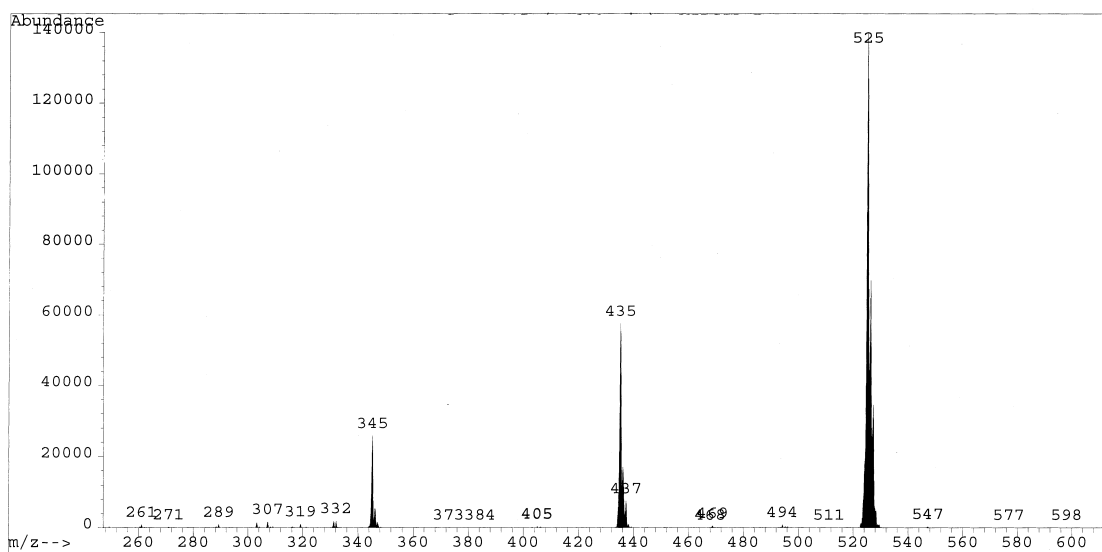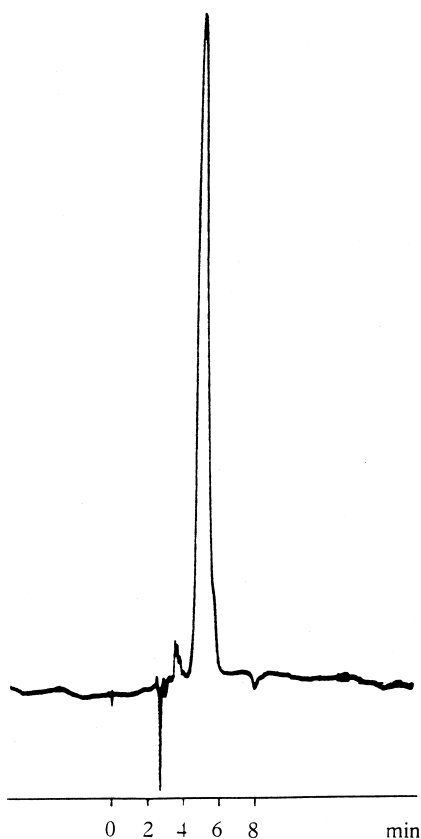
Fig. 3. ES-MS spectrum of α-L-sorbosone.

Fig. 4. HPLC analysis on the carbohydrate column for the synthesized α-L-sorbosone.

in Fig. 3. Relevant ions are m/z 525 $[M+H]^+$, m/z 435 $[M+H-HOSi(CH_3)_3]^+$ and m/z 345 $[M+H-2HOSi(CH_3)_3]^+$. The ES-MS spectra of α-L-sorbosone and derivatives thus showed in all cases the expected information.

Because silica gel caused problems for the chromatographic analysis of α-L-sorbosone, we searched for other chromatographic techniques. On the "Carbohydrate" LC column, the product eluted as one major peak but a little shoulder at the tail is observed (Fig. 4). In analogy with D-glucosone it can be expected that L-sorbosone will decompose in the presence of traces of bases [8]. Therefore the mobile phase was buffered on the acidic side (pH 6.0). Considering the low efficiency it is, however, questionable if LC can offer reliable data. cGC was therefore evaluated. For cGC–MS analysis L-sorbosone was derivatized as O-methyloxime and then persilylated. The analyses on both diphenyl-(5%)dimethylsilicone and on diphenyl(50%)dimethylsilicone showed two main peaks only. Because of *cis/trans* isomerism around the C–N double bonds [9], we expect four peaks but differentiation was not possible.

The chromatogram on the diphenyl(5%)dimethylsilicone column is shown in Fig. 5 and upon library search via the NBS75K library, structures corresponding to D-gluco-hexodialdose were advanced (Fig. 6). This is an acceptable match and indicates
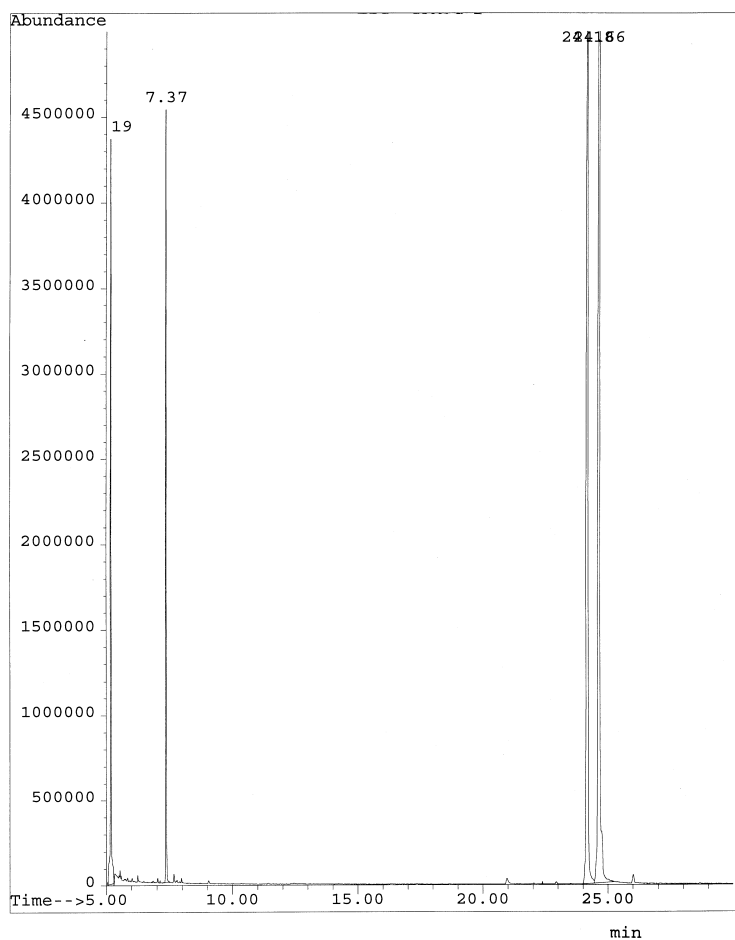
Fig. 5. cGC–MS analysis of the methoximated and persilylated α-L-sorbosone on the diphenyl(5%)dimethylsilicone column.
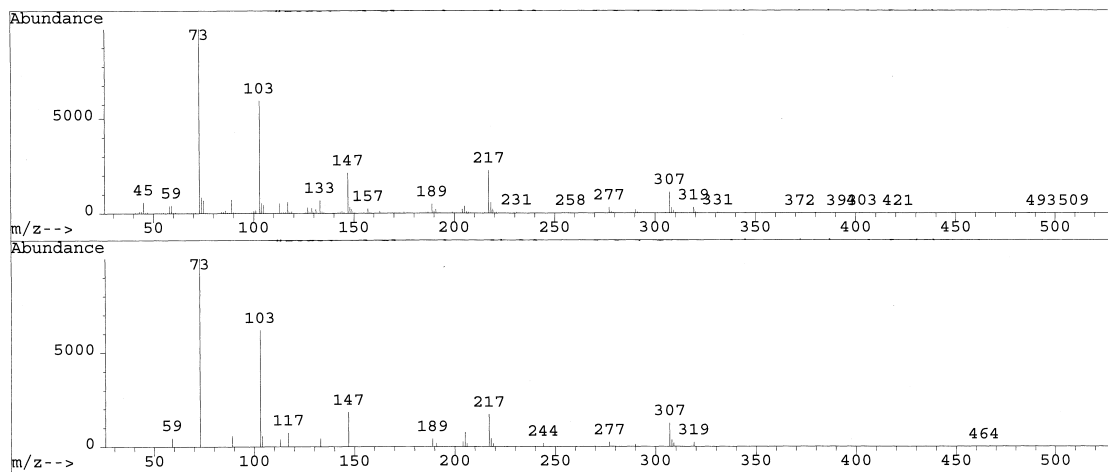

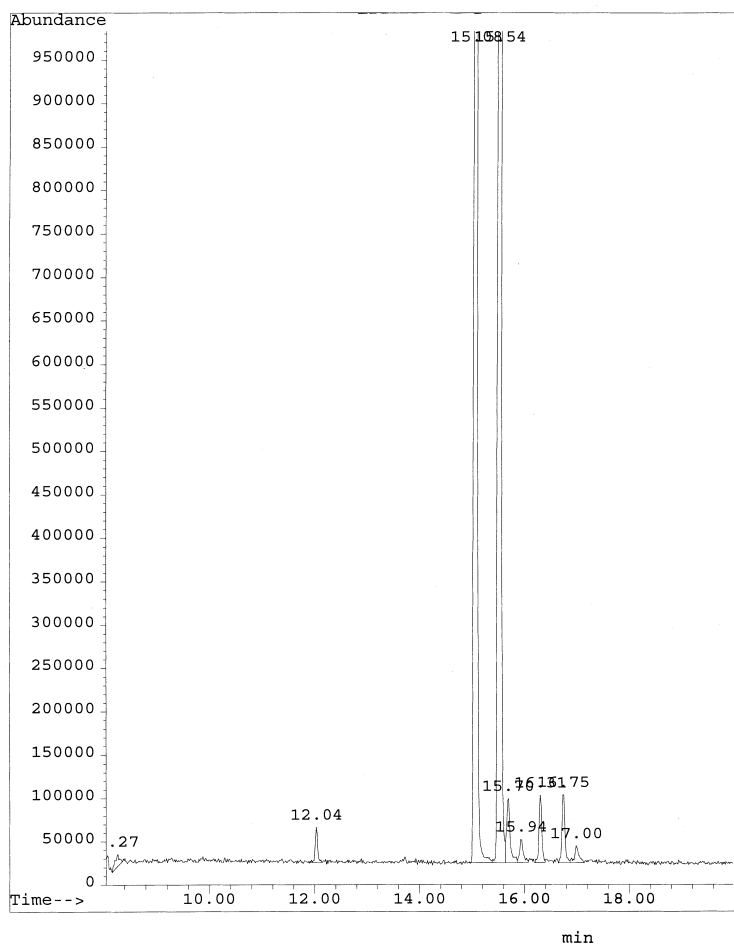Fig. 6. MS spectrum (top) and library search (bottom) for the peak eluting at 24.189 min (first main peak).

Fig. 7. cGC–MS analysis of the methoximated and persilylated α-L-sorbosone on the diphenyl(50%)dimethylsilicone column.

the presence of the two *O*-methyloxime and four trimethylsilyl groups. Complete match cannot be anticipated as the library does not contain α-L-sorbosone. On the other hand, the separation on diphenyl(50%)dimethylsilicone was more complete and revealed some impurities (Fig. 7). The spectra of those were identical as for α-L-sorbosone. Nevertheless, it could be calculated from the cGC–MS trace that the two main compounds account for 97.8% which illustrates the purity of the synthesized α-L-sorbosone.

4. Conclusions

cGC–MS allowed one to determine the purity of samples of α-L-sorbosone obtained via a modified synthetic pathway. The product was unambiguously characterized by ^{13}C NMR spectrometry and ES-MS.

Acknowledgements

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