The Identification of *myo*-Inosose-2 and *scyllo*-Inositol in Mammalian Tissues*

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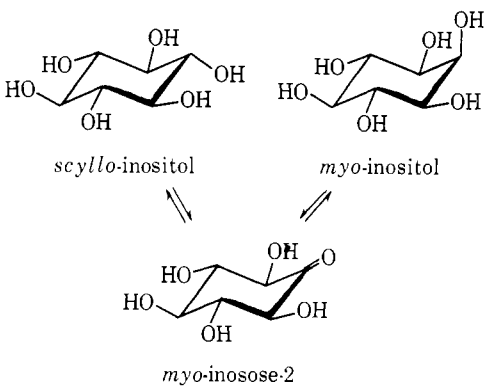
ABSTRACT: *myo*-Inosose-2 has been identified as a micromolar constituent of rat sciatic nerve and calf brain by isolation of its trimethylsilyl (TMS) ether using gas chromatography. TMS-*myo*-inosose-2 was obtained from nerve in nanogram amounts and was identified by two means: (1) hydrolysis to free *myo*-inosose-2, conversion into the oxime, resilylation, and gas chromatographic comparison with authentic TMS-*myo*-inosose-2 oxime; (2) hydrolysis, reduction with sodium amalgam, silylation, and chromatographic identification of the product as TMS-*myo*-inositol. By serial gas chromatography on two types of columns TMS-*myo*-inosose-2 was obtained in microgram amounts from calf brain. The inosose was found to be identical with authentic TMS-*myo*-inosose-2 by combined gas chromatography–mass spectrometry. *scyllo*-Inositol has been found in millimolar concentrations in dog sciatic nerve and rat kidney.

Microgram quantities of TMS-*scyllo*-inositol have been isolated by gas chromatography from these tissues and identified by infrared comparison with authentic TMS-*scyllo*-inositol.

In 1858 Staedler and Frerichs isolated a substance from the kidney, liver, spleen, and gills of elasmobranch fishes which they named scyllite after *Scyllium*, a genus of dogfish. This substance was later found in the flowers of the dogwood, in acorns, in *Cocos* palm leaves, and in other plant tissues. This all-equatorial inositol is today known as scyllitol or *scyllo*-inositol. *scyllo*-Inositol has been observed in mammalian urine (Fleury *et al.*, 1951), in whole rat homogenates (Posternak *et al.*, 1963), and in the locust (Candy, 1967). In 1957 Helleu proposed that *scyllo*-inositol was derived from *myo*-inositol *via* *myo*-inosose-2 as shown. Posternak *et al.* (1963) provided evidence for this pathway by showing that *myo*-inositol was converted into *scyllo*-inositol and *vice versa* by the whole rat. They also found that *myo*-inosose-2 administered to rats was converted into both *myo*- and *scyllo*-inositols. Similar conversions have been observed in plants (Scholda *et al.*, 1964) and in an extract of the locust fat body (Candy, 1967). While *myo*-inosose-2 is produced from *myo*-inositol by strains of *Aerobacter aerogenes* when adapted to the substrate, inosose has never been demonstrated to be a naturally occurring constituent of biological tissues. *scyllo*-Inositol has not been isolated from specific mammalian organs prior to this work.



scyllo-inositol *myo*-inositol

myo-inosose-2

In this paper we describe the isolation and characterization of *myo*-inosose-2 from mammalian nerve and brain and *scyllo*-inositol from nerve and kidney.

Materials and Methods

Tissue Preparation. Dog sciatic nerve (5 g) was incubated under mineral oil for 45 min at 37° in order to reduce the amount of glucose in the sample. This simplified the gas chromatographic collection of *scyllo*-inositol without altering its concentration in the tissues. The tissue was extracted by the following procedure. Water (6 ml/g wet weight of tissue) was added and the sample was heated in a 100° bath for 3 min. This was then homogenized with a glass rod and zinc sulfate was added (0.1 mM/g of tissue) (Somogyi, 1930). The mixture was centrifuged and the deproteinized supernatant taken to dryness. The carbohydrates in the dried extracts were converted into TMS[1] ethers by adding a

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[1] Abbreviations used: TMS, trimethylsilyl; R_g, retention time relative to α-glucose; SE-30, methylsilicone; SE-52, phenylmethylsilicone; EGSS-X, ethylene glycol succinate covalently linked to a methylsilicone; EGS, ethylene glycol succinate; CDMS, cyclohexanedimethanol succinate.

mixture of anhydrous pyridine–hexamethyldisilazane–trimethylchlorosilane (17:2:1, v/v) in a ratio of 1 ml/1 g of tissue (wet weight). The sample was capped, mixed on a Vortex mixer, and stored in a desiccator at room temperature for 48 hr. The preparation was then either chromatographed or stored frozen at −85° at which temperature the TMS ethers were stable for several months.

To prepare *scyllo*-inositol for chromatographic collection from rat kidney, 2.5 g of tissue was extracted directly, without the incubation procedure, deproteinized, taken to dryness, and treated with the trimethylsilylating reagent as before.

In early experiments *myo*-inosose-2 was collected as its TMS ether from rabbit sciatic nerve. Nerves were taken from 6-month-old albino rabbits and frozen at once in liquid nitrogen. Extraction of 0.75 g of tissue, deproteinization, and derivative formation were carried out as before.

To obtain sufficient TMS-*myo*-inosose-2 for mass spectrometry fresh calf brain (600 g) was homogenized in a blender with 1800 ml of water. The homogenate was divided into 300-ml batches and heated for 10 min in a 100° bath. Each batch was then cooled in ice, zinc sulfate was added, the mixture was centrifuged, the supernatant was taken to dryness, then treated with the trimethylsilylating reagent (4 ml/g of original tissue) for 48 hr, and stored at −20° prior to chromatography.

Reagents. Reagent-grade solvents were used throughout the work. Pyridine was dried over Molecular Sieve 5A (Linde Corp.). Hexamethyldisilazane and trimethylchlorosilane were obtained from Applied Science Laboratories, State College, Pa.

Standards. Authentic samples of *myo*-inosose-2 and *scyllo*-inositol were the gift of Professor Laurens Anderson of the University of Wisconsin. The oxime of *myo*-inosose-2 was prepared and converted into the TMS ether according to the general procedure of Sweeley *et al.* (1963). *myo*-Inositol was Fisher Scientific reagent grade.

Gas chromatography was carried out on F & M Models 402 and 810 (F & M Scientific Corp., Avondale, Pa.) using glass columns. The column packings used were purchased precoated (Applied Science Laboratories, State College, Pa.) or coated by evaporation of solutions of the liquid phase in the presence of the support. The following were used: 3% SE-30,[1] 3% SE-52, and 1 or 3% EGSS-X precoated on 80–100 mesh Gas Chrom Q; 20% CDMS ("HiEff-8BP") coated on 60–80 mesh Gas Chrom P; and 15% EGS precoated on 80–100 mesh Gas Chrom P. The EGS and EGSS-X columns were terminated with a 3-in. section of 3% SE-30 on Gas Chrom Q. This reduced the liquid-phase bleed from these columns, thus reducing contamination of collected samples, allowing both columns to be used with an electron-capture detector. Further, the use of the SE-30 terminator reduced the EGSS-X bleed to an insignificant level in the mass spectrometry of *myo*-inosose-2.

The measurement of TMS-*myo*-inosose-2 in unconcentrated tissue samples was carried out with a tritium-foil electron-capture detector (F & M Scientific Corp., Avondale, Pa.). The detector was operated at a pulse interval of 150 μsec, at a temperature of 190° and with 5% methane in argon as carrier (50 cc/min) and purge (125 cc/min). When using the electron-capture detector the trimethylsilylated tissue sample was diluted tenfold in *n*-heptane and 2 μl was injected.

Collection of gas chromatographic fractions for spectrometric identification and for chemical modification was carried out on an F & M Model 402 using glass columns fitted with a stainless-steel effluent splitter of fixed 20:1 ratio. The uncollected portion from the splitter was monitored by a hydrogen flame ionization detector. The detector was operated at 220° with hydrogen and air flow rates of 70 and 350 cc/min, respectively.

Collection and Identification of scyllo-Inositol. The pyridine solutions of the trimethylsilylated nerve and kidney samples were taken nearly to dryness in a stream of nitrogen while heated at 60°. The viscous residues were extracted with carbon tetrachloride, the residue was discarded, and the combined extracts were reduced to one-tenth the original volume of the pyridine solution in a stream of nitrogen. Ten aliquots of 20 μl each were chromatographed on SE-30 (6 ft × 0.25 in.) and the TMS-*scyllo*-inositol was collected in 7 ft × 0.05 in. Teflon tubing. After each collection the tube was rinsed with carbon disulfide. Recovery of *scyllo*-inositol was approximately 75%. Conditions for the chromatography were: column oven 150°, helium carrier flow 100 cc/min, and injection and collection ports 220°.

Standards of TMS-*scyllo*- and -*myo*-inositol for infrared spectrometry were also obtained by collection from the gas chromatograph. Authentic *scyllo*- and *myo*-inositols were converted into their TMS ethers in the usual way, taken to dryness, and reconstituted in carbon tetrachloride to a concentration of 3 μg/μl. The samples were then chromatographed and collected as before.

The pooled samples of standard or tissue-derived material from each chromatographic collection were reduced in volume to approximately 20 μl in a stream of nitrogen. The samples were then transferred by microsyringe into a 0.5-mm path-length sodium chloride cavity cell (Barnes Engineering Company, Stanford, Conn.). The evaporation of the carbon disulfide from the cavity cell rapidly concentrated the sample to the 3 μl of volume of the infrared cell. The infrared spectrum of each sample was obtained using a Perkin-Elmer 127-1271 refracting-beam condenser and a Perkin-Elmer 237-B grating spectrophotometer without solvent compensation. Samples of the collected material were also compared with standards by retention time on EGS and SE-52 columns.

Collection and Identification of myo-Inosose-2. The pyridine solution of the trimethylsilylated rabbit nerve was reduced in volume and extracted as before. The collection was carried out as for TMS-*scyllo*-inositol with a yield of 75 ng of TMS-*myo*-inosose-2. The collected material was rinsed into a glass-stoppered 1-ml centrifuge tube, taken to dryness, and dissolved in 200 μl of 50% aqueous methanol. The methanolic solution was lightly capped with the glass stopper and heated in

an oven at 75° overnight. By this means the TMS ethers were hydrolyzed to the free carbohydrates (Hedgley and Overend, 1960).

Oxime Formation. A portion of the hydrolyzed sample which contained approximately 25 ng of tissue-derived hydroxylamine hydrochloride in 80 μl of anhydrous pyridine for 3 hr at 75°. After cooling 20 μl of hexamethyldisilazane and 10 μl of trimethylchlorosilane were added. This excess of hydroxylamine and trimethylsilylating reagent was found to be necessary in order to carry the reaction to completion. The reaction mixture was allowed to stand at room temperature for 2 days before comparison with standard TMS-*myo*-inosose-2 oxime on SE-52 and EGS columns.

Amalgam Reduction. A second aliquot of the hydrolyzed sample containing about 15 ng of inosose was treated with 2.5% sodium amalgam according to the method of Posternak (1936). The dried sample was taken up in 50 μl of water and 25 μg of amalgam was added followed by 2 μl of 1 N sulfuric acid. When the evolution of hydrogen had stopped a second and third portion of amalgam and sulfuric acid was added. The reaction mixture was then neutralized with 1 N sodium hydroxide and evaporated to dryness in a nitrogen stream at 60°. The residue was extracted with hot methanol, taken to dryness, and treated with 20 μl of pyridine–hexamethyldisilazane–trimethylchlorosilane (10:2:1). The products were compared with authentic TMS-*scyllo*- and -*myo*-inositol on an EGS column.

Collection of TMS-myo-Inosose-2 for Mass Spectrometry. The pyridine solution of trimethylsilylated calf brain extract (see Tissue Preparation) was reduced in volume on a rotary evaporator first at water pump pressure, then under high vacuum. The evaporation was carried out at room temperature and terminated when the residue was a viscous yellow oil. The oil was dissolved in *n*-heptane and centrifuged to remove a heavy precipitate. The precipitate was extracted twice with heptane and the combined solutions were reduced in volume to 1% of the original volume of the pyridine solution. Each collection was carried out by the injection of 100 μl of the clear yellow heptane solution onto a 5 ft × 12 mm o.d. glass column packed with 20% CDMS. The column oven was maintained at 190° and the injection block at 270°. Under these conditions the 100-μl injection did not alter the efficiency of the column, which remained at 600 theoretical plates for this and smaller sample sizes. The injection size was such that the constituent of greatest concentration (TMS-*myo*-inositol) did not overload the column. The level of inosose was insufficient to be detected by the hydrogen flame detector monitoring the splitter (5% of the sample). Therefore the collection was made over a wide portion of the chromatogram known to center on the retention time of TMS-*myo*-inosose-2. The effluent was collected in disposable Pasteur pipets at room temperature. The pipets were rinsed out with *n*-heptane and the samples were pooled. After five–ten injections the CDMS column lost efficiency, probably due to contamination of the column with nonvolatile residues, and had to be repacked.

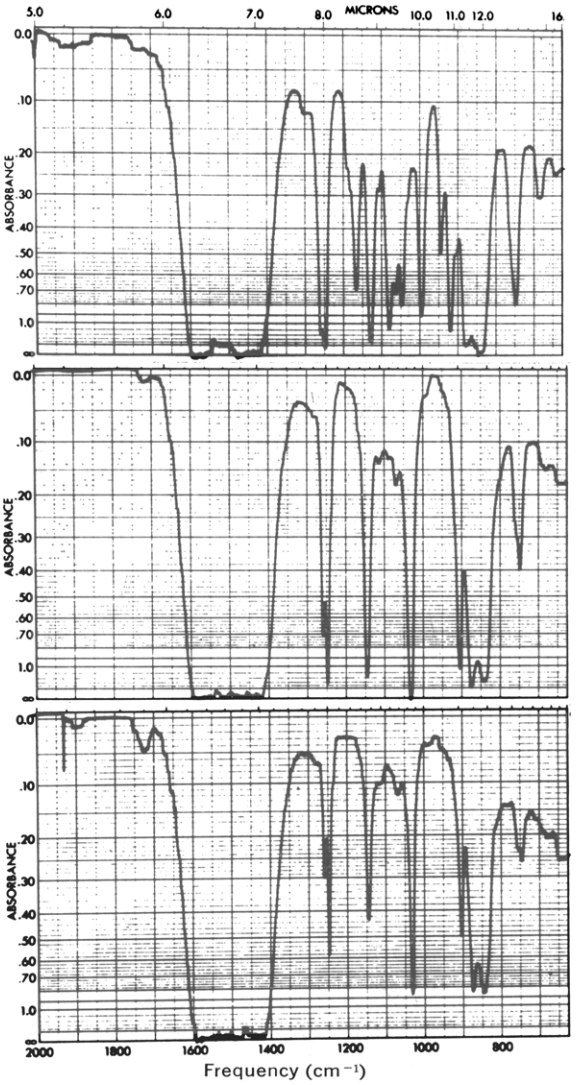

FIGURE 1: Infrared spectra of TMS-*myo*-inositol (top). TMS-*scyllo*-inositol (center), and TMS-*scyllo*-inositol from dog sciatic nerve (bottom). Each spectrum was obtained with approximately 25 μg of sample dissolved in 3 μl of carbon disulfide and contained in a 0.5-mm path-length cell as described under Materials and Methods. At an absorbance of 0.1 the solvent absorbs from 1660 to 1360 cm^{-1}, from 890 to 825 cm^{-1}, and from 675 to 625 cm^{-1}.

The pooled, collected material from the CDMS column was analyzed using the electron-capture detector and the heptane volume was adjusted to a TMS-*myo*-inosose-2 concentration of 1 μg/100 μl of solution. This solution was injected in 100-μl aliquots onto a 5 ft × 12 mm o.d. glass column packed with 3% SE-30. The column was maintained at 170° and the injection port at 250°. The TMS-inosose was collected as before, only over a narrower range, corresponding to the theoretical foot and tail of the material. The collection was dissolved in a small volume of *n*-heptane and stored at

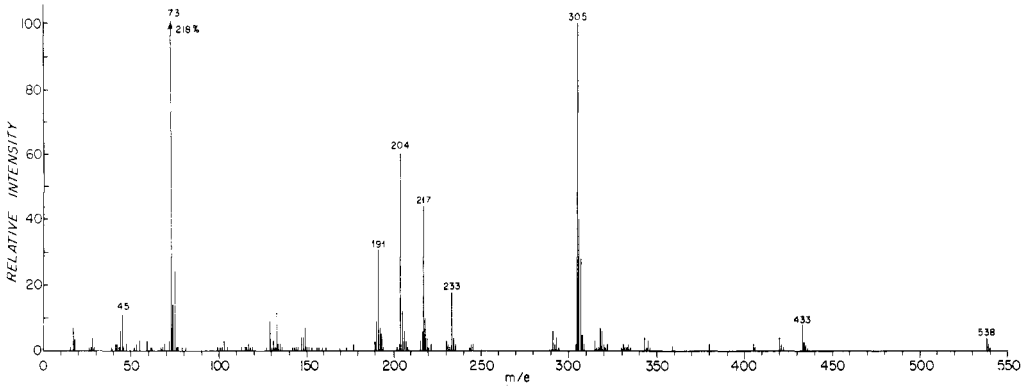

FIGURE 2: Bar graph calculated from the spectrum of authentic TMS-*myo*-inosose-2. The most abundant ion fragment at *m/e* 73 ($[CH_3]_3Si^+$) is not taken as the base peak. The ion fragment at 305 is used instead (see Discussion). Instrumental conditions are given under Materials and Methods.

−85° until sufficient material for mass spectrometry was available.

Mass spectrometry was carried out on an LKB 9000 combined mass spectrometer–gas chromatograph[2] (LKB Produkter, Stockholm, Sweden). Chromatography was performed on a 6 ft × 0.25 in. glass column packed with 3% EGSS-X terminated with 3 in. of 3% SE-30. The spectrometer was operated at 70 eV and 3500 V ionizing and accelerating potentials. The heptane solution of TMS carbohydrates collected from the SE-30 column was adjusted in volume until the TMS-*myo*-inosose-2 was present at a concentration of 1 μg/5 μl. Mass spectra were obtained following the injection of 5 μl of this sample. The spectrum of TMS-inosose was taken by an 8-sec scan across the center of the eluting inosose peak. The mass spectrum so obtained was compared with that of authentic TMS-*myo*-inosose-2 chromatographed both as the pure compound and mixed with TMS α- and β-glucose and TMS-*myo*-inositol. Integral masses were obtained by reference to perfluorokerosene. The bar graph (Figure 2) was prepared using as base peak the fragment second in abundance to *m/e* 73 ($[CH_3]_3Si^+$).

Results

scyllo-Inositol. When chromatographed on SE-30, TMS-*scyllo*-inositol has a retention time, R_g,[1] of 2.19, well resolved from TMS-β-glucose (R_g 1.77) and TMS-*myo*-inositol (R_g 2.69). Using this column we were able to collect that TMS-*scyllo*-inositol efficiently and free of contaminants. Both tissues provided material which gave a single peak when chromatographed on EGS and on SE-52. The retention times on these columns were in each case identical with that of standard TMS-*scyllo*-inositol.

The infrared spectrum of the material collected from dog sciatic nerve is shown in Figure 1 to be identical with the spectrum of authentic TMS-*scyllo*-inositol. The spectrum of the substance collected from rat kidney was also identical with that of authentic TMS-*scyllo*-inositol. Figure 1 also compares the infrared spectra of TMS-*scyllo*- and -*myo*-inositols. The considerable differences between the spectra of such closely related compounds strengthens our view that the spectrum of TMS-*scyllo*-inositol is unique and that the identity of the tissue collected material is rigorous. The simplification of the spectrum of the trimethylsilyl ether of *scyllo*-inositol with respect to that of *myo*-inositol is consistent with a structure in which all hydroxyls are in an equivalent configuration (equatorial). Absorption bands which are present in TMS-*myo*-inositol[3] but not in TMS-*scyllo*-inositol occur at 1170, 1130, 1080, 1049, 995, 945, 920, and 680 cm^{-1}.

myo-Inosose-2. When the sample collected from rat nerve was examined it was found that TMS-sorbitol had been collected with TMS-*myo*-inosose-2, presumably by

[2] Mass spectrometry was carried out at the University of Pittsburgh Graduate School of Public Health through the generosity of Professor C. C. Sweeley.

[3] Recently Loewus (1966) reported the synthesis of crystalline 1,2,3,4,5,6-hexakis-*O*-(trimethylsilyl)-*myo*-inositol. Loewus described the infrared spectrum of this method in a KBr disk as follows: "five strong absorptions in the 950–1200-cm^{-1} region at 995, 1045, 1075, 1115, and 1180 cm^{-1}." Since our gas chromatograph collected material differs in its CS_2 solution spectrum from that of Loewus' KBr spectrum we have synthesized the crystalline TMS-*myo*-inositol according to Loewus using *myo*-inositol purchased from the Pfanstiehl Inc., Waukegan, Ill. The melting point of the TMS-*myo*-inositol was 119–120° (uncor), within 1° of Loewus. The infrared spectrum of this material in CS_2 solution was identical with the spectrum we previously obtained (Figure 1). A KBr disk prepared from this material absorbed at 990, 1045, 1065, 1085, 1130, and 1170 cm^{-1}, a spectrum very similar to that of the CS_2 solution. We do not believe that the differences between our spectra and that of Loewus are due to sample impurities since we obtained the same spectrum from two sources of *myo*-inositol. Rather we believe the differences are due to the variations commonly seen in KBr disks as a result of crystal modifications and methods of disk preparation. In all cases, solution spectra are more reproducible and the CS_2 spectrum presented here is thought to be correct for this substance.

tailing. After hydrolysis and treatment with hydroxylamine the resilylated mixture was chromatographed on EGS and compared with authentic TMS-*myo*-inosose-2 oxime. The chromatogram showed that the tissue sorbitol remained unchanged, while a product of the same retention time as that of TMS-*myo*-inosose-2-oxime had been formed. Chromatography on SE-52 also supported the identity of the tissue inosose oxime with authentic material. As a further means of establishing the identity of the TMS-*myo*-inosose-2 collected from rabbit nerve the hydrolyzed material was treated with sodium amalgam. Posternak (1936) has shown that amalgam reduction of *myo*-inosose-2 produces both *scyllo*- and *myo*-inositols. After trimethylsilylation our amalgam-reduced tissue inosose was chromatographed on EGS. In addition to unchanged sorbitol, a peak corresponding to TMS-*myo*-inositol was observed. A barely detectable peak at the correct retention time for TMS-*scyllo*-inositol was also present.

The collection of TMS-*myo*-inosose-2 from 600 g of calf brain was complicated by the large volumes of derivatized tissue it was necessary to chromatograph in order to obtain sufficient TMS-*myo*-inosose-2 for mass spectrometry. Attempts to sublime the TMS derivatives from dried residues of the trimethylsilylating reagent resulted in 80% losses of the TMS-inosose. The best procedure for the first stage of purification was found to be injection of the heptane extract directly onto the CDMS column. This resulted in the rapid buildup of nonvolatile residues in the injection port and a gradual decrease in column efficiency. With freshly packed columns 60% of the injected inosose could be recovered. The material collected from the CDMS column was free of the nonvolatile components and free of substances eluting before TMS-fructose and after TMS-*myo*-inositol. The other carbohydrates in the collected material were present in approximately their original relative tissue concentrations. The pooled material from the CDMS column was then rechromatographed on SE-30. The material collected from this column was still a mixture, with the TMS-*myo*-inosose enriched 2300-fold with respect to TMS-*myo*-inositol. While the original calf brain contained 0.36 μg/g (wet weight) of *myo*-inosose-2 and 1.0 mg/g of *myo*-inositol, in the collected sample the ratio was 0.82 mg of TMS-*myo*-inosose-2 to 1.0 mg of TMS-*myo*-inositol. Also present in this sample were the TMS ethers of α- and β-glucose, *myo*-inositol, an unidentified substance presently under study, and minor amounts of other substances.

A portion of the SE-30-collected material containing 1 μg of TMS-*myo*-inosose-2 was chromatographed on 3% EGSS-X at the inlet of the mass spectrometer. When the spectrum obtained in this way was compared with that of authentic TMS-*myo*-inosose-2 they were both found to possess a molecular ion at *m/e* 538, as shown in Figure 3. Minor differences were observed in other areas of the spectrum of the pure standard (Figure 2) when this was compared with the spectrum of TMS-inosose in the tissue mixture. However, when authentic TMS-inosose contained in a mixture with pure TMS-α- and β-glucose and TMS-*myo*-inositol was chromatographed under identical conditions, the mass spectrum of the inosose was identical with that obtained from the tissue. The differences observed in the spectra thus appear to be due to incomplete chromatographic separation.

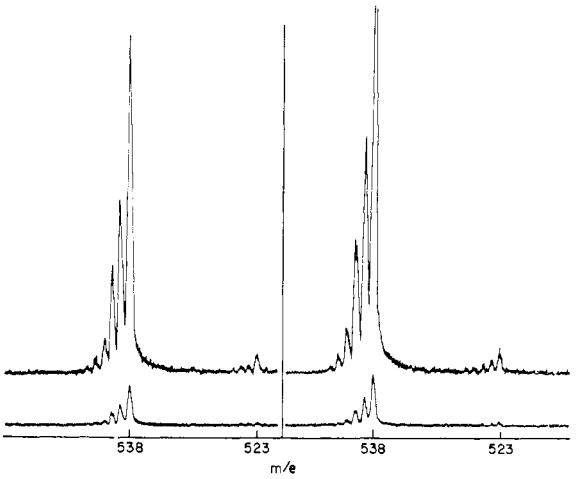

FIGURE 3: A portion of the mass spectrum of authentic pentakis(trimethylsilyl)-*myo*-inosose-2 (left) and the TMS-*myo*-inosose-2 collected from calf brain. The molecular ion is at *m/e* 538 with silicon isotope contributions as follows (*m/e*, abundance as per cent of the 538 peak): 539, 51; 540, 32; 541, 10; 542, 5; 543, 1.4. The theoretical abundance of the M + 1 peak is 49.7% considering ^{2}H, ^{13}C, ^{17}O, and ^{29}Si. The M − 15 peak at *m/e* 523 appears to have the same ratio of 523:524 and thus is a simple loss of methyl. Gas chromatographic column bleed makes no contribution at these masses. Instrumental conditions are given under Materials and Methods.

Discussion

This work provides the first evidence for the natural occurrence of *myo*-inosose-2 and the first isolation of *scyllo*-inositol from mammalian organs. The simultaneous occurrence of *scyllo*-inositol, *myo*-inosose-2, and *myo*-inositol in a variety of mammalian organs and their tissue concentrations will be reported elsewhere (Sherman *et al.*, 1968).

We have established that *myo*-inosose-2 is not an artifact formed during the trimethylsilylation of *myo*-inositol. When a standard preparation of TMS-*myo*-inositol was chromatographed and examined with an electron-capture detector, no TMS-*myo*-inosose-2 could be detected. The detection limit in this experiment was one part in 10^{7}. While the possibility exists that nonspecific dehydrogenases in tissues might act on *myo*-inositol to produce inosose, it would be impossible to distinguish these from a *myo*-inositol dehydrogenase without purification of the enzyme.

It is our belief that *myo*-inosose-2 is formed in one of

two ways. Dehydrogenases have been found in the locust (Candy, 1967) which reduce *myo*-inosose-2 to *myo*-inositol or to *scyllo*-inositol using different cofactors. Presumably this reaction could be reversed to produce inosose from the inositols. Alternatively, *myo*-inosose-2 might arise from an intermediate in the cyclization of glucose-6-P to *myo*-inositol-1-P (Chen and Charalampous, 1966). Such an intermediate is *myo*-inosose-2-(1-P) which Kindl (1966) has postulated for this pathway. Phosphatase action could produce the inosose observed.

The mass spectrum of pentakis(trimethylsilyl)-*myo*-inosose-2 (Figures 2 and 3) deserves separate comment. While the results in this paper were obtained on a medium-resolution mass spectrometer and definitive assignments are not possible, a few of the fragments observed may be tentatively identified by their silicon content. Figure 3 shows that the ^{29}Si and ^{30}Si isotopes make a considerable contribution from M + 1 through M + 6. While fragments greater than M + 1 become difficult to correlate with silicon content, M + 1 provides a simple check of the number of silicon atoms in a proposed structure.

As a first approximation it might be expected that TMS-*myo*-inosose-2 would give rise to ions similar to those obtained from cyclohexanone (Williams *et al.*, 1964). One of the major fragments obtained from cyclohexanone is $\cdot CH_2CH_2CH_2^+$. The analogous structure from TMS-*myo*-inosose-2 would be TMSOCHCH-(OTMS)C^+HOTMS, mass 306. The base peak in Figure 2 is at 305, and might have the structure TMSOCH=C^+(OTMS)=CHOTMS. A similar fragment is observed in the spectrum of cyclohexanone. Unfortunately it is not possible to corroborate this structure by the abundance of the M + 1 peak. If the fragment ion at *m/e* 305 has three silicon atoms the ^{13}C–^{29}Si contribution to 306 is 29%. The remaining 11% observed may be due to the fragment analogous to that obtained from cyclohexanone.

The other fragment ions observed do not seem to be the counterparts of those seen with cyclohexanone. Ions in Figure 2 which, by isotope ratio, have two silicon atoms are at *m/e* 233, 217, 204, and 191. These may be rationalized by the loss, from the *m/e* 306 fragment, of the elements of TMS, TMSO, TMSOCH, and $TMSO(CH)_2$.

The ion fragment at *m/e* 433 has the correct abundance at *m/e* 434 to contain four silicon atoms and thus appears to result from the loss of CH_3 and TMSOH from the molecular ion.

Sharkey *et al.* (1957) report the formation of ion fragments from TMS alcohols which arise by the loss of CH_2, *e.g.*, $(CH_3)_2SiOH$ (*m/e* 75) and CH_3SiH_2 (*m/e* 45). While fragments do occur at these *m/e* values, no other fragments have been recognized in Figure 2 which derive from the loss of mass 14.

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