Carbohydrate Research, 129 (1984) 21–32
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

ANALYSIS OF *O*-METHYLINOSITOLS BY GAS–LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY*

RONALD G. BINDER AND WILLIAM F. HADDON

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Albany, California 94710 (U.S.A.)

(Received August 16th, 1983; accepted for publication in revised form, November 9th, 1983)

ABSTRACT

A procedure for identifying natural *O*-methylinositols by g.l.c.–m.s. analysis is described. Chromatographic retention-index values for these compounds and commonly co-occurring compounds are given. The procedure is illustrated by analyses of clover and peanut cyclitols.

INTRODUCTION

Although methyl ethers of inositols occur in many plant families[1] and sometimes in amounts greater than saccharides—(+)-pinitol, for example, is the major soluble carbohydrate in soybean[2,3]—no comprehensive method of analysis for them is available.

In recent years, *O*-methylinositols have usually been analyzed by g.l.c. of their trimethylsilyl (Me_3Si) ether derivatives: bornesitol in *Cannabis sativa*[4]; pinitol in legume seeds[5–7], legume foliage[2,3,8–10], soybean nodules[11,12], Cycad plants[10] and *Picea* and *Pinus*[13–15]; quebrachitol in *Cannabis sativa*[4] and maple syrup[16]; sequoyitol in *Pinus* species[13,14] and Cycad plants[10]; and *O*-methyl-*scyllo*-inositol in mung beans and chick peas[6]. Relative retention-times on XE-60, OV-1, and OV-17 for Me_3Si ethers of five naturally occurring isomers have been published[4,17]. Such information is particularly helpful because samples of most of the inositol methyl ethers are not readily available.

Lack of standards for comparison has limited identification by g.l.c.–m.s. Mass spectra of bornesitol, pinitol, quebrachitol, and sequoyitol are published[18], but not for the Me_3Si ethers, except for pinitol[2,3,15]. Consequently, there have been occasions when only tentative identification could be made[2,3,13,14].

We have obtained the natural mono-*O*-methylinositols (**3a** is enantiomeric with the natural isomer[19]) and also 2-*O*-methyl-*myo*-inositol. Relative retention-

*Reference to a company and/or product name is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

times of Me_3Si derivatives on a polar and a nonpolar column and the mass spectra have been determined.

EXPERIMENTAL

General methods. — Procedures to remove noncyclitol compounds from aqueous plant extracts included treatment with cation- and anion-exchange resins, fermentation of sugars, chromatography on cellulose (Whatman standard grade) with 6:1 acetone–water as eluting solvent, and chromatography on Sephadex G-10. Trimethylsilyl ethers were prepared with hexamethyldisilazane and chlorotrimethylsilane in pyridine. After removal of pyridine on a rotary evaporator, the residue was taken up in heptane and filtered. Routine analyses were performed on a column (5.5 m × 0.32 cm) of 2% Silar 10C on 60–80 Chromosorb W at 180°.

Isolation or preparation of cyclitols. — *1L-1-O-Methyl*-myo-*inositol* [L-(+)-*bornesitol*] (**1a**). This compound was provided by Prof. Loewus, Washington State University. (Cyclitol structures are shown in Fig. 1.)

2-O-Methyl-myo-*inositol* (**1b**). *myo*-Inositol in barium hydroxide solution was treated with dimethyl sulfate[20] to give methyl ethers, including **1b**. Compounds soluble in hot ethanol were acetylated with acetic anhydride in pyridine. The concentration of **1b** pentaacetate was increased by crystallization of **1b** pentaacetate from ethanol or from acetone at −20°. The **1b** recovered from this compound after saponification had m.p. 210.5–211.5° (lit.[20] m.p. 210–211°).

1D-4-O-Methyl-myo-*inositol* [D-(+)-*ononitol*] (**1c**). Ononitol was isolated from alfalfa (*Medicago sativa*)[21]. A sample crystallized from ethanol had m.p. 171.5° (lit.[22] m.p. 172°) and $[\alpha]_D^{20}$ +6.55° (water) (lit.[22] $[\alpha]_D$ +6.6° ±1°).

5-O-Methyl-myo-*inositol* [*sequoyitol*] (**1d**). Sequoyitol was isolated from *Ginkgo biloba* leaves[23]. A sample crystallized from methanol had m.p. 240–241° (lit.[23] m.p. 239°).

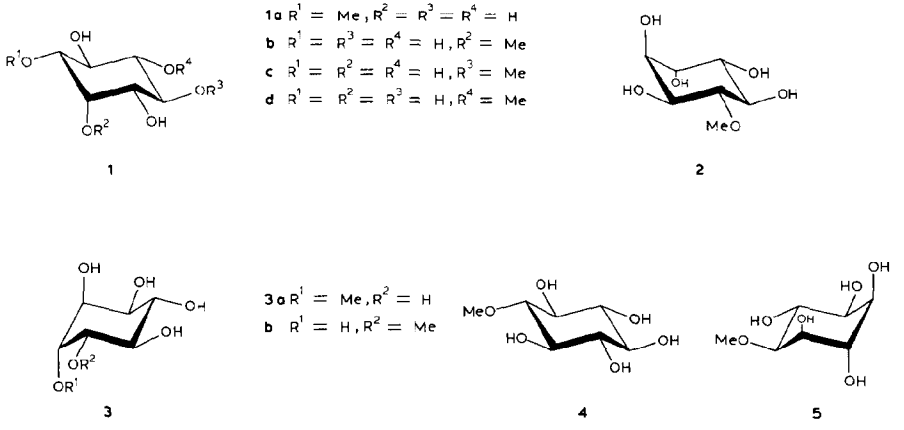

Fig. 1. Structures of *O*-methylinositols.

*1*D-*3*-O-*Methyl*-chiro-*inositol* [D-(+)-*pinitol*] (**2**). Pinitol was isolated from pine sawdust[24,25]. A sample crystallized from ethanol had m.p. 187–189° (lit.[25] m.p. 185–186°).

*1*L-*1*-O-*Methyl*-chiro-*inositol* (**3a**). A mixture containing **3a** and pinitol was prepared[26]. Isopropylidene acetals were synthesized[27,28] and partitioned between water and chloroform (in which di-*O*-isopropylidenepinitol is soluble). The water solution was acidified and heated to hydrolyze any isopropylidene acetal. Solvent was evaporated and the residue was trimethylsilylated. The Me_3Si ether of **3a** crystallized from methanol at −20°.

*1*L-*2*-O-*Methyl*-chiro-*inositol* [L-(−)-*quebrachitol*] (**3b**). A sample of quebrachitol, provided by Mr. William Butler of Plantation Division Uniroyal, was purified by treatment of an aqueous solution with activated carbon and crystallization from water.

O-*Methyl*-scyllo-*inositol* (**4**). Compound **4** was isolated from mung bean seeds[29]. A sample crystallized from methanol had m.p. 243–244° (lit.[29] m.p. 243°).

*1*D-*1*-O-*Methyl*-muco-*inositol* (**5**). A mixture of **5** and pinitol was isolated from redwood (*Sequoia sempervirens*) needles by generally following the procedures of Dittrich *et al.*[30]. However, contrary to earlier results[30], pinitol was eluted before **5** during chromatography on a column (2.5 × 100 cm) of Dowex 1-X4 in the borate form with mM sodium borate solution as eluent. After removal of borate, **5** was obtained as a noncrystallizing liquid having $[\alpha]_D^{25}$ −11.8° (ethanol). The literature value $[\alpha]_D^{22}$ −53.2° is for a not extensively purified, synthetic sample[31]. However, after chromatography on Sephadex G-10 and formation of and recovery from the Me_3Si ether derivative, **5**, with high purity indicated by g.l.c., did not show a lower rotation.

1-D-chiro-*Inositol.* This compound was obtained by demethylation of pinitol with hydrogen iodide.

scyllo-*Inositol.* This compound was obtained by demethylation of *O*-methyl-*scyllo*-inositol with hydrogen iodide.

Clover cyclitols. Trifolium repens plants were homogenized in methanol. After removal of methanol from the solution, the residue was allowed to ferment for two days following the addition of dry yeast. The resulting mixture was treated with activated carbon, filtered, passed through a column of cation-exchange resin, and then titrated to pH 7 with anion-exchange resin. Chromatography on Sephadex G-10 provided a fraction containing cyclitols.

Peanut cyclitols. An aqueous extract of commercial raw Virginia peanut was fermented for 16 h. Calcium oxide was added and the mixture kept for 1 h near 100°. It was then made acid with phosphoric acid and heated for 1 h, whereupon it was made neutral with calcium oxide and centrifuged. After treatment of the solution with active carbon, water was evaporated off. The residue was subjected to silylating reagents and cyclitol Me_3Si ethers were recovered.

G.l.c.–m.s. — Mass spectra were obtained by using a VG 70/70 HS magnetic mass spectrometer (VG-Analytical Ltd., Altrincham, Cheshire, U.K.) with a VG-

2000 data system, and an HP model 5830A gas chromatograph (Hewlett Packard, Inc., Avondale, PA). Two g.l.c. columns were used in the course of the g.l.c.–m.s. work, one a 42 m × 0.5 mm i.d. OV-101 support-coated open tubular (SCOT) column (Scientific Glass Engineering Pty., Ltd., North Melbourne, Australia) and the other a 15 m × 0.32 mm i.d. fused silica (FS) capillary column coated to 0.25-μm film thickness with 077-methyl silicone (Quadrex Corp., New Haven, CT). Both columns were interfaced directly to the mass spectrometer; the FS column extended directly into the ion source via an 0.5-mm (i.d.) glass re-entrant jet (VG-Analytical Ltd., P/N 03058A)[32]. The temperature program for the SCOT column was 80° for 0.5 min, 30°/min to 170°, and then 3°/min to 220° and for the FS column 60° for 0.5 min, 30°/min to 170°, and then 3°/min to 220°. The injection temperature was 220° and the carrier gas was helium at a linear velocity of 100 cm/sec.

G.l.c. retention-index values. — Kovats retention-index (r.i.) values[33,34] were established during g.l.c.–m.s. as follows: injection of an *n*-alkane mixture (C_{16}–C_{23}) prior to analysis of a set of samples provided an r.i. retention-time (or mass-spectral scan number) correlation that was linear to ±1 r.i. unit from C_{18}–C_{22}. R.i. values of unknown peaks in subsequent chromatograms were determined by linear interpolation between expected retention-times (scan numbers) for the *n*-alkanes. Co-injection of two alkane standards (usually C_{17} and C_{23}) as internal references compensated for small run-to-run differences in the r.i.-scan number correlation.

RESULTS AND DISCUSSION

Mass spectra. — Fig. 2 shows the 70-eV e.i. spectra of the Me_3Si ethers of three *O*-methyl inositols (**1b**, **1c**, and **2**) and of the Me_3Si ether of *chiro*-inositol, one of the inositols that frequently co-occurs with the *O*-methylinositols in natural products. Table I gives acccurate abundance-values for the diagnostically important peaks of the *O*-methylinositol spectra for all nine isomers, and Table II lists the elemental composition of the more-abundant ions, which were assigned from accurate mass measurement of **1a** at high resolution.

As indicated in Fig. 2, mass spectra readily differentiate *O*-methylinositols from the structurally similar inositol Me_3Si ethers, based on the occurrence of ions at *m/z* 89, 159, 247, 260, 374, 375, and 449, which are absent or of minor importance in the spectra of the eight inositol isomers[35]. In each case, these characteristic ions of the *O*-methylinositols retain the OCH_3 group and lie 58 mass units below corresponding peaks in the spectra of the inositols. The elemental-composition difference for these pairs of ions is C_2H_6Si, as confirmed by the compositional assignments given in Table II. For example, ions at *m/z* 432 and 507 are common to the two sets of compounds; in the *O*-methylinositol spectra their formation involved loss of CH_3OH at some stage of genesis. They are isomeric and possibly structurally identical to equivalent ions formed by loss of Me_3SiOH in the spectra of the inositols. Elemental-composition assignments for peaks common to the *O*-methylinositol and inositol Me_3Si ethers (Table II) were consistent in every case

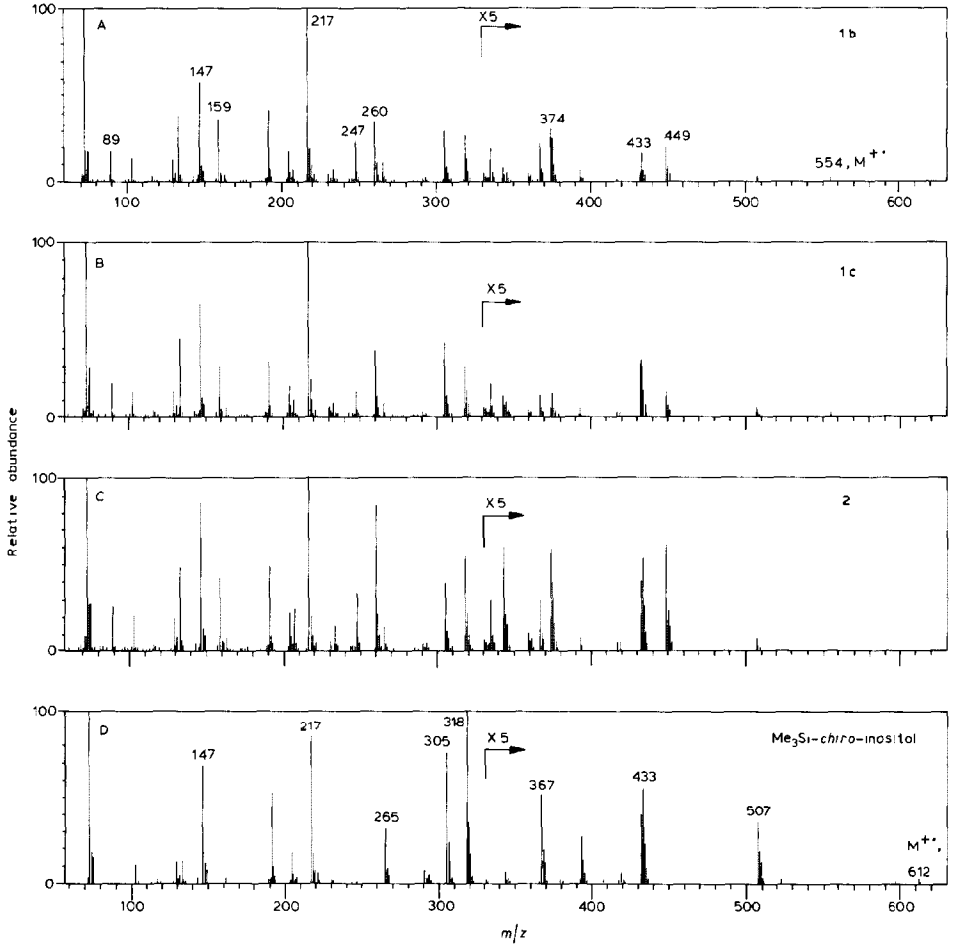

Fig. 2. E.i. mass spectra (70 eV) of cyclitol Me_3Si ethers. Source temperature, 180°.

with the ion-structure postulates for the inositols established previously from deuterium labeling by Sherman[35].

The high-mass ions of the *O*-methylcyclitol spectra probably retain the integrity of the ring, as indicated by the dependence of ion abundance on configuration. For peaks above *m/z* 300, eleven of the fifteen peaks indicated in Table I vary more than 3-fold in relative abundance (r.a.) for the different isomers; ten of thirteen peaks show comparable variation for the eight inositols[35]. The abundance data do not correlate with specific equitorial–axial relationships, in agreement with Sherman's observations for the inositols. However, the nine isomers may be identified from mass spectra alone using r.a. values at *m/z* 374, 375, 432, 433, and 449. Variations between different mass spectrometers may, of course, be considerable[15] and such identification requires spectra of very high quality.

TABLE I

70-eV MASS SPECTRA OF TRIMETHYLSILYL ETHERS OF *O*-METHYLINOSITOLS[a]

Compound	m/z													
	73	89	129	133	147	159	191	204	207	217	247	260	265	305
1a	234	20	15	47	70	46	42	21	10	100	20	18	7.7	43
1b	191	9.5	12	56	57	26	30	18	5.2	100	44	18	8.0	18
1c	214	18	13	38	57	36	41	18	7.0	100	23	35	11	30
1d	232	19	15	45	65	29	32	17	9.7	100	14	38	6.2	43
2	321	26	19	48	85	41	48	22	24	100	34	84	14	38
3a	276	16	15	55	74	42	40	21	12	100	35	77	12	47
3b	306	26	19	54	73	34	46	22	14	100	38	54	21	45
4	265	18	12	45	57	25	50	36	6.2	100	29	72	5.9	29
5	254	19	17	42	54	34	36	18	11	100	37	48	8.2	33

Compound	m/z													
	318	343	367	374	375	393	432	433	449	464	507	522	539	554
1a	24	1.0	3.4	3.0	3.1	1.4	2.1	3.7	2.6	0.20	0.59	—	0.15	0.50
1b	17	0.55	3.8	1.9	2.4	0.97	1.6	1.1	2.0	0.29	0.42	0.05	0.12	0.67
1c	27	1.5	4.3	6.0	5.0	1.1	0.86	3.3	4.0	0.21	0.57	—	0.19	0.35
1d	29	2.5	2.3	1.1	2.5	0.9	6.1	6.5	2.8	0.07	0.72	0.04	0.12	0.48
2	54	12	5.7	12	7.8	1.1	8.0	11	12	0.30	1.3	0.20	0.23	0.18
3a	21	1.8	5.3	4.2	4.8	1.8	2.0	2.6	4.6	0.17	1.2	—	0.14	0.34
3b	43	2.9	9.6	4.6	5.7	1.8	4.7	7.7	9.1	0.17	1.1	—	0.25	0.20
4	50	1.1	2.6	1.3	1.6	1.9	1.4	3.2	3.0	0.10	0.9	0.02	0.06	0.29
5	35	4.1	3.6	0.65	2.2	0.8	0.8	2.4	4.7	0.07	0.63	—	0.10	0.28

[a]Ion-source temperature, 200°.

TABLE II

PARTIAL HIGH-RESOLUTION MASS SPECTRUM OF PENTA-*O*-(TRIMETHYLSILYL)QUEBRACHITOL ($C_{22}H_{54}O_6Si_5$)

Measured m/z	*Assigned elemental composition*	*Error, p.p.m.*
449.2045	$C_{18}H_{41}O_5Si_4$	3.3
433.2080	$C_{18}H_{41}O_4Si_4$	−0.4
432.1969	$C_{18}H_{40}O_4Si_4$	−8.0
375.1857	$C_{16}H_{35}O_4Si_3$	3.6
374.1796	$C_{16}H_{34}O_4Si_3$	8.2
367.1636	$C_{13}H_{35}O_4Si_4$	6.4
335.1509	$C_{13}H_{31}O_4Si_3$	−6.4
318.1477	$C_{13}H_{30}O_3Si_3$	−8.1
305.1415	$C_{12}H_{29}O_3Si_3$	−3.2
265.1110	$C_9H_{25}O_3Si_3$	−0.7
260.1254	$C_{11}H_{24}O_3Si_2$	−3.9
247.1184	$C_{10}H_{23}O_3Si_2$	−0.6
217.1069	$C_9H_{21}O_2Si_2$	−3.7
207.0856	$C_7H_{19}O_3Si_2$	−8.2
204.1016	$C_8H_{20}O_2Si_2$	6.8
191.0931	$C_7H_{19}O_2Si_2$	4.1
159.0836	$C_7H_{15}O_2Si$	−3.2
147.0655	$C_5H_{15}OSi_2$	−4.0

TABLE III

KOVATS RETENTION-INDEX VALUES AND RETENTION TIMES RELATIVE TO HEXA-*O*-(TRIMETHYLSILYL)-*myo*-INOSITOL FOR TRIMETHYLSILYL ETHERS OF *O*-METHYLINOSITOLS AND RELATED COMPOUNDS

Compound	*Retention index*		*Relative retention-times*
	FS[a]	*SCOT*[b]	*Silar 10C*[c]
1*D*-1-*O*-Methyl-*muco*-inositol (**5**)	1860	—	0.349
D-Fructose	1864	1855	0.329
D-(+)-Pinitol (**2**)	1880	1885	0.306
D-Galactose	1910 (d)	—	0.626
L-(−)-Quebrachitol (**3b**)	1911	1918	0.349
D-Glucose	1937	1934	0.595 (0.773)
1*L*-1-*O*-Methyl-*chiro*-inositol (**3a**)	1947	—	0.479
D-(+)-Ononitol (**1c**)	2000	2008	0.543
Sequoyitol (**1d**)	2008	2025	0.577
chiro-Inositol	2011	2022	0.530
O-Methyl-*scyllo*-inositol (**4**)	2025	1998	0.488
L-(+)-Bornesitol (**1a**)	2043	2047	0.689
2-*O*-Methyl-*myo*-inositol (**1b**)	2072	—	0.863
scyllo-Inositol	2090	—	0.671
myo-Inositol	2143	2155	1.000

[a]Fused silica column (15 m) coated with methyl silicone. [b]Support-coated, open tubular column (42 m) coated with OV-101. See Experimental for temperature-program data. [c]Column (5.5 m × 0.32 cm) of 2% Silar 10C on Chromosorb W, at 180°: Retention times measured from beginning of heptane peak. [d]Peaks also at r.i. 1954 and 1878.

Gl.c.–m.s. retention-index values. — The generally low abundance (<10% r.a.) of structurally diagnostic peaks complicates identification of the *O*-methyl inositols at low levels in natural-product extracts. Measurement of g.l.c. retention-index values concurrently with g.l.c.–m.s. analysis alleviates this problem considerably by providing a second, readily determined structure-related parameter. For example, the mass spectra of **1c** and **3a** are very similar; identifying these compounds from mass spectra alone requires accurate determination of *m/z* 432 (0.86 per cent r.a. for **1c**; see Table I). However, **1c** and **3a** are readily distinguished by their r.i. values of 1947 and 2000. Conversely, for ononitol and sequoyitol (**1c** and **1d**), which have much closer r.i. values of 2000 and 2008 on the FS column, the ion-abundance ratio *m/z* 374:432 differs by a factor of 40.

Table III shows the r.i. values on the two high-resolution methyl silicone columns and retention times relative to *myo*-inositol Me_3Si ether on the Silar 10C column. Variations in r.i. between the SCOT and FS capillary columns (same stationary phase) are about the same as reported for urinary acid Me_3Si ethers on equivalent columns[36]. The maximum difference is 0.27 r.i., for *O*-methyl-*scyllo*-inositol (**4**). In the two methyl silicone columns, the elution order of **1c**, **1d**, *chiro*-inositol, and **4** differs. In the absence of reference compounds, accurately determined mass spectra would be needed for verification of identity. However, indications of compound identities may conveniently be obtained by comparison of chromatograms from a methyl silicone column and a Silar 10C column. For routine g.l.c.–m.s.

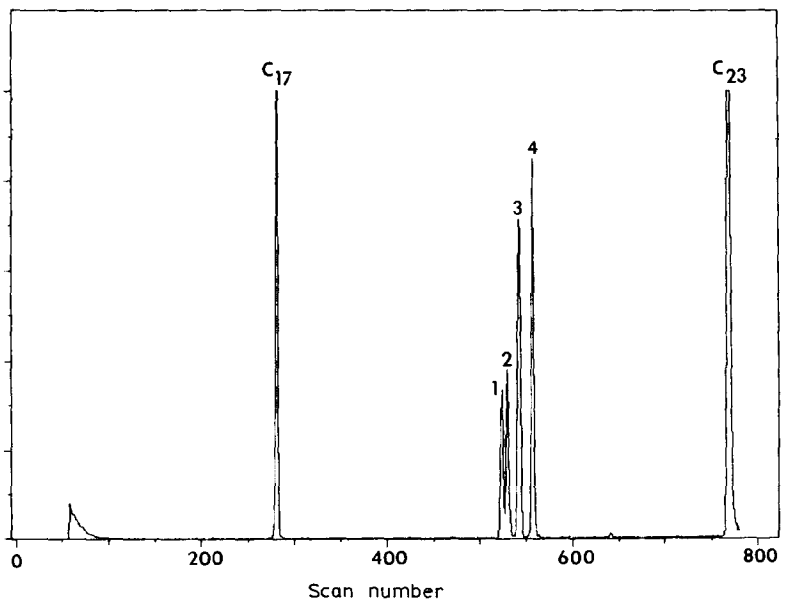

Fig. 3. Separation of *O*-methylinositol Me_3Si ethers on a 15-m methyl silicone fused-silica column: 1, **1c**, r.i. = 2000; 2, **1d**, r.i. = 2008; 3, **4**, r.i. = 2025; 4, and **1a**, r.i. = 2043. See Experimental section for g.l.c. conditions.

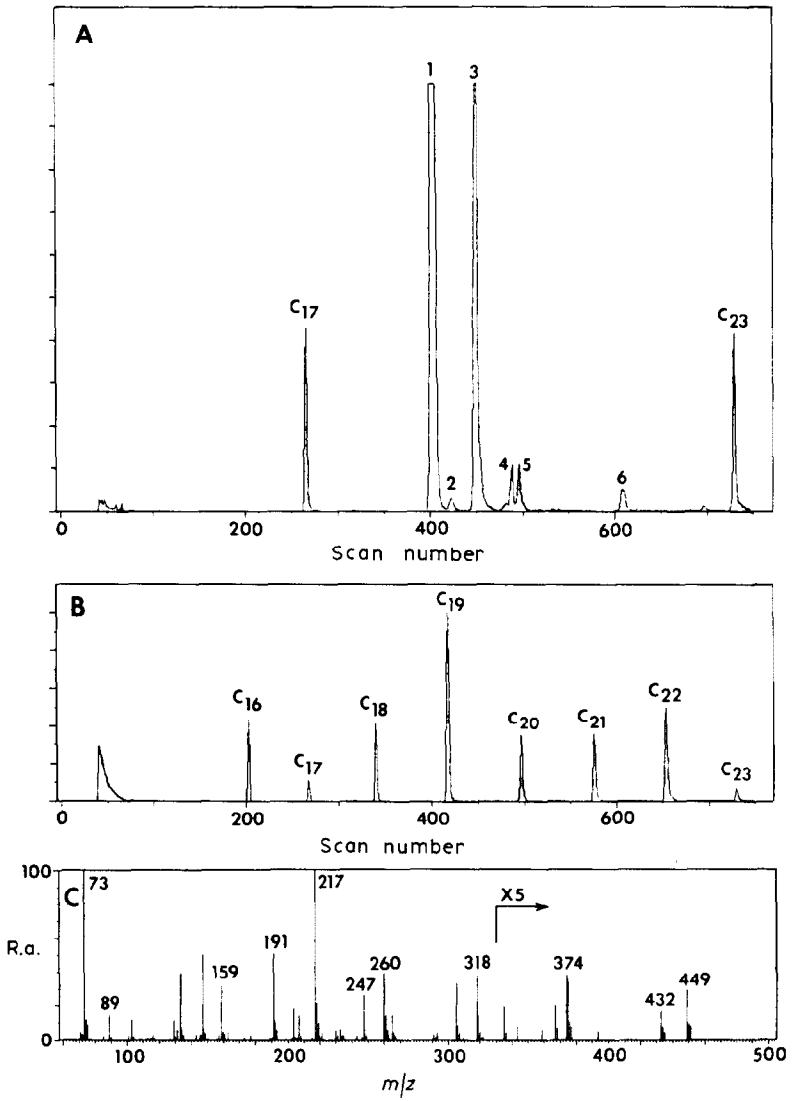

Fig. 4. G.l.c.–m.s. analysis of cyclitols in clover: A, t.i.c. chromatogram, including C_{17} and C_{23} *n*-alkane internal standards; B, T.i.c. chromatogram of r.i. calibration mixture, C_{16}–C_{23} *n*-alkanes, and C, e.i. mass spectrum of peak 5, ononitol (**1b**) Me_3Si ether.

work, the FS column is superior to the SCOT column in chromatographic resolutions and convenience, as expected[37].

Fig. 3 illustrates the separation of the four *O*-methylinositols (**1c**, **1d**, **4**, and **1a**) that have similar r.i. values on the FS column. The attained chromatographic resolution of 8 r.i. units for 10% valley between adjacent peaks provides full separation of ononitol and sequoyitol (**1c** and **1d**), but would fail to resolve sequoyitol

and *chiro*-inositol; here, however, spectral differences readily distinguish the two compounds, as discussed previously.

Values of r.i. given in Table III have remained constant over months on the same g.l.c. column. Calibration for r.i. *vs.* scan number at the beginning of a series of samples and inclusion of an *n*-alkane internal-standard with each sample (see Experimental) routinely provides ±3 r.i. units or better accuracy for assignment of

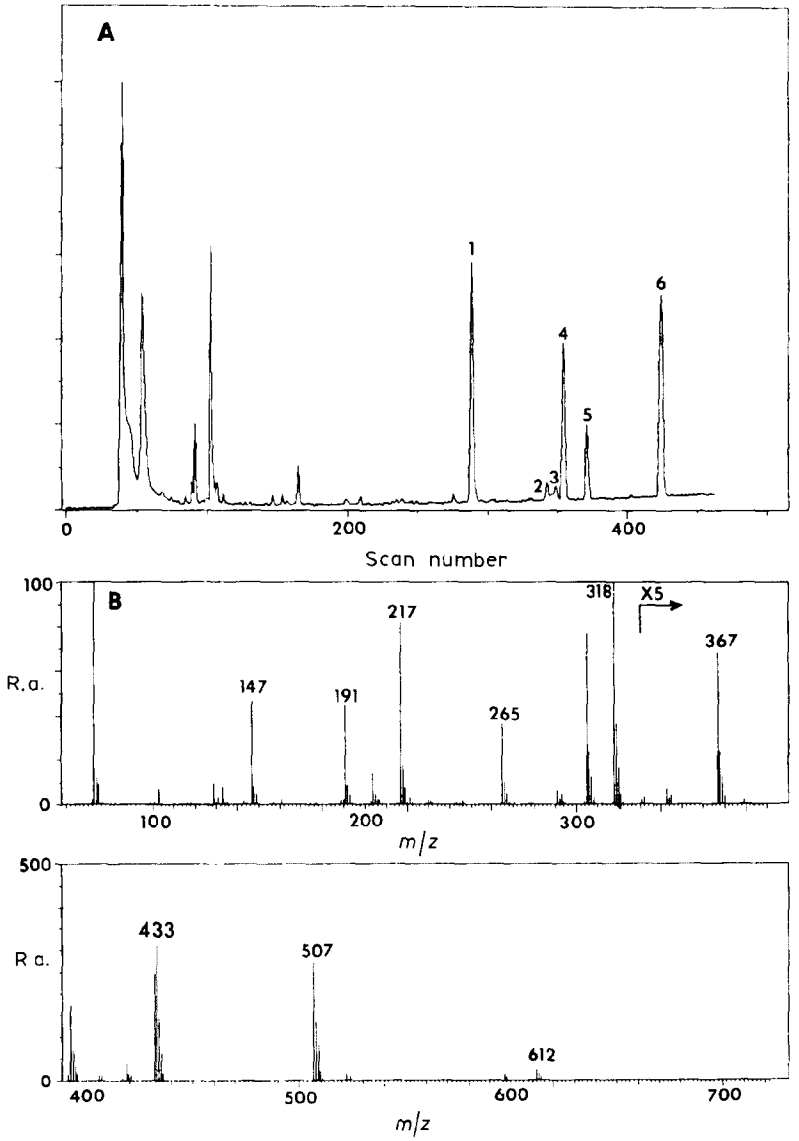

Fig. 5. G.l.c.–m.s. analysis of cyclitols in Virginia peanuts: A, t.i.c. chromatogram; B, e.i. mass spectrum of peak 4, *chiro*-inositol Me_3Si ether.

unknown peaks, about the same accuracy as reported in a recent statistical evaluation of Kovats-index values in systems based on linear temperature-program operation[38] of the g.l.c. For the capillary and SCOT columns, the r.i. values are the same for both g.l.c.-alone and g.l.c.–m.s. conditions.

Examples. — Use of capillary g.l.c.–m.s. facilitates identification of cyclitols over a wide concentration range in the same extract. The data of Fig. 4 illustrates this capability for a cyclitol-containing mixture isolated from clover and chromatographed on the FS column after purification and trimethylsilylation (see Experimental). Fig. 4A is the total ion-current (t.i.c.) chromatogram for this mixture and Fig. 4B is the t.i.c. trace for the *n*-alkane r.i. calibration-mixture obtained separately. Peaks 1, r.i. = 1881, 5, r.i. = 2000, and 6, r.i. = 2142, correspond by agreement of r.i. and mass spectrum to pinitol, ononitol, and *myo*-inositol, respectively. The concentration of ononitol is <0.5% of pinitol based on t.i.c. peak areas; the mass spectrum of peak 5 (Fig. 4C) confirms the identification by comparison of peak intensities at *m/z* 374, 375, 432, and 433 with the reference spectra of ononitol and sequoyitol (see Figs. 1a,b). Likewise, comparisons of spectra eliminate quebrachitol (**3c**) and glucose as candidates for peaks 2 and 3 of this sample. The mass spectrum of peak 3 (r.i. = 1939) indicates methyl β-D-glucopyranoside as a probable structure.

Fig. 5 shows the g.l.c.–m.s. results from analysis of an extract of Virginia peanuts on the SCOT column. This mixture contains three *O*-methylcyclitols and two inositols. Peaks 1, 3, and 5 (r.i. = 1882, 2008, and 2052) correspond to pinitol, ononitol, and bornesitol and peaks 4 and 6 (r.i. = 2020 and 2156) indicate *chiro*-inositol and *myo*-inositol, based on agreement of r.i. and mass spectra. The spectrum of peak 4 (r.i. = 2020), shown in Fig. 5B, identifies this component as *chiro*-inositol rather than sequoyitol (r.i. = 2025). Except for *myo*-inositol, none of these compounds has previously been reported as a constituent of peanuts.

In the g.l.c.–m.s. analysis, choice of a non-polar methyl silicone stationary-phase for gas chromatography was made for compatibility with other g.l.c.–m.s. projects undertaken currently, rather than to provide optimal separation of carbohydrate Me_3Si ethers. Other liquid phases, such as the Silar 10C employed for packed-column work, may be preferable. However, the methyl silicone columns resolved all but one pair of the target compounds, sequoyitol and *chiro*-inositol, a pair readily distinguished from the mass spectra even at low levels. Kovats r.i. values remained constant for over a year on the SCOT column, even with extensive use of this column for g.l.c.–m.s. analysis of unrelated samples. There was no need to re-measure Kovats r.i. values of the standards after the initial determination. The constancy of these values allowed measurements to be carried out intermittently in a g.l.c.–m.s. facility shared among multiple projects.

ACKNOWLEDGMENTS

We thank Sandra J. Tillin for assistance in obtaining mass spectra and Professor Frank Loewus and Mr. William Butler for samples of cyclitols.

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