Retention Data in GLC Analysis; Carbohydrate-Related Hydroxy Carboxylic and Dicarboxylic Acids as Trimethylsilyl Derivatives

Göran Petersson, Chalmers University of Technology, Department of Engineering Chemistry, Fack, S-402 20 Göteborg, Sweden

Abstract

Retention index (I) and relative retention are given for trimethylsilyl esters/ethers of approximately 170 acids and lactones. The structural categories studied are aldonic (hydroxy monocarboxylic) acids, aldaric (hydroxy dicarboxylic) acids, non-carbohydrate (mainly dicarboxylic) acids, and lactones of aldonic acids. Data are given for methyl, phenyl (50%), and trifluoropropyl (50%) silicone stationary phases.

Diagrams of the retention index on the non-polar phase versus the increase in the retention index on either of the other phases are useful for qualitative analysis. The dependence of retention on structure is interpreted in terms of non-polar and polar interactions between solute and stationary phase. Structural units of the solutes are attributed retention index increments which permit predictions of retention from structure or structure from retention.

The reference retention index data can be used in temperature-programmed analysis if retentions are measured by "methylene unit" (MU) values. Differences between the I and MU concepts are discussed.

Introduction

A large variety of hydroxy acids are of great importance both in organic chemical and in metabolic processes, particularly in connection with the degradation of carbohydrates. Silylation combined with gas chromatography is now an established technique for the analysis of these compounds.

The early methods, based on the pioneering work of Sweeley et al. (1), involved silylation after evaporation of water from acidic aqueous solutions. This approach was useful for particular types of acids, e.g., certain aldonic acids, and is still frequently used. However, formation of isomeric lactones, incomplete lactonization, and formation of intermolecular esters may cause multiple GLC peaks and quantitative errors. These disadvantages can be avoided by initial conversion of the acids (including lactones) to anions and subsequent preparation of mixed ester and ether trimethylsilyl (Me_3Si) derivatives for GLC analyses (2). This technique permits convenient analysis of virtually all structural classes of hydroxy acids.

Publications, each containing retention data for more than a dozen compounds on several stationary phases, have appeared for aldonic (2,3) and deoxyaldonic (2) acids, aldaric acids (4), and aldonolactones (5,6). All of these report relative retentions in isothermal analysis, as do numerous other publications which give fewer data.

The purpose of the present work is to present more versatile retention data for a comprehensive collection of silylated hydroxy acids. The intention is also to demonstrate how such data can be used to obtain the maximum qualitative information in both isothermal and temperature-programmed work.

Experimental

The investigated acids were either obtained commercially or isolated in work published from this department. The Me_3Si derivatives were prepared according to previously described methods (2). Mass spectrometry was used to confirm the structure of the derivatives.

The retention data were determined on Perkin-Elmer Model 900, 990 and 3920 gas chromatographs with flame ionization detectors. A system with two parallel packed columns was used on P.-E. 3920 for simultaneous temperature-programmed analysis on two stationary phases. The gas stream was split after the injector, and separate detectors, amplifier units, and recorders were used for the two channels.

Experimental details which are closely related to the retention data are given in connection with the tables.

Isothermal Retention Data

Retention index (I) and relative retention (r) on three widely used types of silicone stationary phases are given in Tables I-IV for the Me_3Si derivatives of all compounds included in this study.

Investigated Compounds

In selecting compounds, the aim was a comprehensive collection of unsubstituted hydroxy acids related to monosaccharides. Acids containing aldehydo or keto groups were not included because these acids are often reduced or converted to oximes before analysis.

The hydroxy monocarboxylic (aldonic) acids are gathered in Table I and the hydroxy dicarboxylic (aldaric) acids in Table II. Among these acids there is a bias towards oxidative and alkaline degradation products of carbohydrates because of the research interests in this department. The collection of aldonolactones (Table IV) emphasizes 1,4-lactones which are normally the predominant isomers. Furthermore, analysis of aldonic acids as lactones often involves acidic lactonization (5-7) which favours the formation of 1,4-lactones. Lactones of aldaric acids have not gained analytical importance and were not included in this study. The non-carbohydrate acids (Table III) include primarily compounds having retention data comparable with those of the aldonic and aldaric acids. The alkanedioic and alkenedioic acids occur together with the lower hydroxy dicarboxylic acids in many analytical applications. The esterdimeric acids are formed during lactonization if the corresponding monomeric acids (which are eluted much earlier) are present.

Table I. Aldonic Acids[a]

Acid [c]	100% Me silicone (OV-1, 160°) I	r [b]	50% Ph, 50% Me (OV-17, 160°) I	r [b]	50% $C_2H_4CF_3$, 50% Me (QF-1, 120°) I	r [b]
Acyclic acids						
Glyceronic (glyceric)	1340	0.052	1341	0.092	1500	0.086
2-C-Methylglyceronic	1348	0.054	1314	0.082	1460	0.072
2-Deoxytetronic	1448	0.084	1446	0.146	1616	0.145
3-Deoxytetronic	1431	0.078	1430	0.136	1589	0.129
4-Deoxyerythronic	1364	0.058	1346	0.094	1512	0.091
4-Deoxythreonic	1376	0.061	1358	0.099	1514	0.092
Erythronic	1581	0.151	1536	0.218	1699	0.212
Threonic	1589	0.156	1568	0.251	1723	0.237
3,4-Dideoxypentonic	1517	0.114	1529	0.211	1693	0.206
3,5-Dideoxy-erythro-pentonic	1422	0.075	1411	0.125	1570	0.118
3,5-Dideoxy-threo-pentonic	1437	0.080	1436	0.140	1597	0.133
3-Deoxy-2-C-(hydroxymethyl)tetronic	1671	0.224	1626	0.326	1789	0.319
2-C-Methylerythronic	1626	0.184	1563	0.246	1730	0.244
2-Deoxy-erythro-pentonic	1698	0.252	1659	0.379	836	0.395
2-Deoxy-threo-pentonic	1697	0.251	1655	0.373	1834	0.392
3-Deoxy-erythro-pentonic	1683	0.236	1660	0.380	1824	0.375
3-Deoxy-threo-pentonic	1698	0.252	1681	0.419	1848	0.418
Ribonic	1823	0.438	1749	0.569	1915	0.565
Arabinonic	1835	0.463	1777	0.646	1940	0.632
Xylonic	1818	0.428	1767	0.617	1917	0.570
Lyxonic	1832	0.457	1760	0.599	1936	0.621
2,4-Dideoxy-3-C-methylpentonic (mevalonic)	1575	0.147	1575	0.260	1749	0.266
2,6-Dideoxy-ribo-hexonic	1726	0.286	1672	0.402	1852	0.425
3,4-Dideoxyhexonic [d]	1802	0.400	1787	0.676	1969	0.720
3,6-Dideoxy-ribo-hexonic	1710	0.266	1669	0.396	1840	0.403
3,6-Dideoxy-arabino-hexonic	1717	0.275	1684	0.425	1861	0.442
3-Deoxy-2-C-hydroxymethyl-erythro-pentonic	1931	0.707	1860	0.942	2043	1.003
3-Deoxy-2-C-hydroxymethyl-threo-pentonic	1919	0.670	1849	0.896	2027	0.933
2-C-Methylribonic	1880	0.564	1791	0.689	1976	0.742
2-C-Methylarabinonic	1876	0.554	1786	0.674	1966	0.710
2-Deoxy-arabino-hexonic	1915	0.659	1853	0.912	2044	1.007
2-Deoxy-lyxo-hexonic	1920	0.673	1858	0.935	2050	1.035
3-Deoxy-ribo-hexonic	1920	0.673	1861	0.946	2044	1.007
3-Deoxy-arabino-hexonic	1921	0.676	1867	0.971	2043	1.004
3-Deoxy-xylo-hexonic	1915	0.659	1872	0.995	2048	1.026
3-Deoxy-lyxo-hexonic	1910	0.644	1849	0.894	2036	0.973
6-Deoxymannonic	1865	0.528	1771	0.629	1959	0.687
6-Deoxygalactonic	1899	0.615	1826	0.809	1993	0.801
3-O-Methylgulonic	1938	0.728	1882	1.038	2040	0.990
2-C-(Hydroxymethyl)pentonic [e]	2035	1.118	1920	1.235	2107	1.333
Allonic	2035	1.115	1927	1.276	2119	1.410
Altronic	2061	1.247	1968	1.531	2143	1.568
Gluconic	2068	1.286	1973	1.569	2153	1.639
Mannonic	2034	1.113	1923	1.254	2115	1.385
Gulonic	2029	1.087	1924	1.256	2103	1.309
Idonic	2082	1.368	1996	1.743	2176	1.812
Galactonic	2062	1.256	1972	1.561	2143	1.570
Talonic	2059	1.235	1964	1.507	2155	1.656
Anhydro acids						
2,5-Anhydro-3,4-dideoxypentonic	1456	0.087	1625	0.325	1872	0.465
1,4-Anhydro-3-deoxypentitol-2-carboxylic [d]	1652	0.206	1690	0.436	1868	0.456
2,5-Anhydrogluconic	1898	0.612	1926	1.271	2189	1.928
2,5-Anhydromannonic	1842	0.477	1870	0.986	2120	1.416
2,5-Anhydrotalonic	1890	0.590	1921	1.241	2171	1.776

[a] Columns: 2 m × 0.2 cm i.d.; stainless steel. Packings: 0.5% OV-1 on 100/120 mesh Chromosorb G; 0.5% OV-17 on 100/120 mesh Chromosorb G; 3% DC QF-1 on 100/120 mesh Gas Chrom Q. Carrier gas: ~ 30 ml/min purified nitrogen.

[b] Relative retention relating to the Me_3Si derivative of glucitol (~ 12 min for OV-1; ~ 7 min for OV-17; ~ 15 min for QF-1).

[c] Arranged in order of (1) increasing number of skeleton C atoms, (2) increasing number of hydroxyl (including methoxyl) groups and (3) increasing carbon chain length.

[d] Either or both of the erythro and threo isomers.

[e] Either or both of the xylo and lyxo isomers.

Table II. Aldaric Acids[a]

Acid	100% Me silicone (OV-1, 160°)		50% Ph, 50% Me (OV-17, 160°)		50% $C_2H_4CF_3$, 50% Me (QF-1, 120°)	
	I	r	I	r	I	r
Glyceraric (tartronic) [b]	1386	0.064	1455	0.152	1675	0.190
C-Methylglyceraric	1364	0.058	1362	0.101	1554	0.110
Deoxytetraric (malic)	1494	0.103	1530	0.212	1762	0.283
O-Methylerythraric	1554	0.134	1617	0.313	1823	0.372
O-Methylthrearic	1574	0.146	1646	0.358	1878	0.478
C-(Hydroxymethyl)glyceraric	1583	0.152	1576	0.261	1754	0.273
Erythraric	1613	0.174	1617	0.313	1812	0.354
Threaric	1658	0.212	1688	0.432	1875	0.471
C-Ethylglyceraric	1437	0.080	1440	0.142	1602	0.136
2-Deoxy-3-C-methyltetraric	1488	0.100	1499	0.185	1700	0.213
2,3-Dideoxypentaric	1583	0.152	1631	0.333	1883	0.489
2,4-Dideoxypentaric	1583	0.152	1629	0.331	1873	0.467
C-(2-Hydroxyethyl)glyceraric	1696	0.250	1694	0.444	1884	0.491
2-Deoxy-3-C-(hydroxymethyl)tetraric	1714	0.271	1705	0.466	1922	0.582
2-Deoxy-erythro-pentaric	1720	0.278	1728	0.517	1948	0.655
2-Deoxy-threo-pentaric	1730	0.291	1750	0.573	1974	0.736
3-Deoxy-erythro-pentaric	1721	0.280	1736	0.538	1954	0.673
3-Deoxy-threo-pentaric	1733	0.295	1758	0.593	1985	0.774
2-O-Methylxylaric	1781	0.364	1825	0.805	2020	0.905
3-O-Methylarabinaric	1776	0.357	1808	0.744	2003	0.840
Ribaric	1867	0.532	1843	0.873	2050	1.037
Arabinaric	1862	0.522	1841	0.864	2030	0.945
Xylaric	1872	0.545	1868	0.978	2050	1.034
2,4-Dideoxy-3-C-methylpentaric	1612	0.173	1645	0.356	1870	0.461
2,3,4-Trideoxyhexaric	1682	0.235	1737	0.539	1999	0.823
C-(3-hydroxypropyl)glyceraric	1771	0.348	1780	0.657	1961	0.693
3,4-Dideoxyhexaric [c]	1839	0.471	1869	0.982	2118	1.401
C-(2,3-Dihydroxypropyl)glyceraric	1949	0.767	1928	1.278	2113	1.368
3-Deoxy-2-C-hydroxymethyl-erythro-pentaric	1963	0.816	1941	1.357	2191	1.940
3-Deoxy-2-C-hydroxymethyl-threo-pentaric	1940	0.737	1910	1.179	2134	1.504
2-Deoxy-arabino-hexaric	1952	0.777	1927	1.277	2143	1.565
2-Deoxy-lyxo-hexaric	1972	0.847	1958	1.467	2168	1.751
3-Deoxy-ribo-hexaric	1939	0.732	1930	1.290	2141	1.550
3-Deoxy-arabino-hexaric	1949	0.767	1952	1.425	2158	1.675
3-Deoxy-xylo-hexaric	1948	0.761	1934	1.317	2153	1.639
3-Deoxy-lyxo-hexaric	1956	0.789	1959	1.470	2163	1.713
3-O-Methylgularic	1988	0.909	1979	1.610	2170	1.770
C-(erythro-1,2,3-trihydroxypropyl)glyceraric	2072	1.310	2004	1.801	2169	1.762
C-(threo-1,2,3-trihydroxypropyl)glyceraric	2084	1.377	2016	1.904	2177	1.822
Allaric	2060	1.242	1987	1.669	2187	1.904
Altraric	2091	1.419	2037	2.098	2219	2.195
Glucaric	2070	1.295	2005	1.814	2189	1.920
Mannaric	2026	1.074	1939	1.346	2118	1.401
Idaric	2119	1.602	2078	2.518	2256	2.589
Galactaric	2104	1.500	2055	2.272	2234	2.347

[a] Columns, retention data and ordering of the acids as in Table I.

[b] The systematic name is preferred since the ending "onic" in tartronic implies a monocarboxylic acid in carbohydrate nomenclature.

[c] Either or both of the erythro and threo isomers.

Table III. Non-Carbohydrate Acids[a]

Acid	100% Me silicone (OV-1, 160°)		50% Ph, 50% Me (OV-17, 160°)		50% $C_2H_4CF_3$, 50% Me (QF-1, 120°)	
	I	r	I	r	I	r
Carbocyclic hydroxy acids						
Shikimic	1844	0.482	1871	0.990	2053	1.050
Quinic	1918	0.668	1851	0.906	2006	0.852
Esterdimeric hydroxy acids[b]						
(Hydroxyethanoyl)hydroxyethanoic	1383	0.063	1501	0.187	1723	0.237
2-Hydroxypropanoyl-2-hydroxypropanoic	1376	0.061	1456	0.153	1667	0.183
3-Hydroxypropanoyl-3-hydroxypropanoic	1567	0.142	1688	0.432	1966	0.709
2-Hydroxybutanoyl-2-hydroxybutanoic	1515	0.113	1593	0.281	1793	0.326
Alkanedioic acids						
Ethanedioic (oxalic)	1100	0.018	1181	0.046	1475	0.077
Propanedioic (malonic)	1174	0.025	1257	0.064	1515	0.092
Methylpropanedioic	1208	0.029	1264	0.066	1492	0.083
Butanedioic (succinic)	1291	0.042	1380	0.109	1616	0.145
Dimethylpropanedioic	1192	0.027	1226	0.056	1457	0.071
Ethylpropanedioic	1275	0.039	1330	0.088	1558	0.112
Methylbutanedioic	1307	0.045	1373	0.106	1613	0.143
Pentanedioic (glutaric)	1386	0.064	1476	0.167	1728	0.242
2,2-Dimethylbutanedioic	1317	0.047	1367	0.103	1586	0.127
meso-2,3-Dimethylbutanedioic	1360	0.057	1404	0.121	1647	0.167
2-Methylpentanedioic	1407	0.070	1484	0.173	1725	0.239
3-Methylpentanedioic	1413	0.072	1493	0.180	1741	0.257
Hexanedioic (adipic)	1490	0.101	1586	0.272	1849	0.419
3,3-Dimethylpentanedioic	1431	0.078	1489	0.177	1714	0.227
Heptanedioic	1592	0.158	1692	0.441	1955	0.677
Octanedioic	1691	0.245	1797	0.708	2059	1.075
Nonanedioic	1793	0.384	1898	1.117	2164	1.718
Alkenedioic acids						
cis-Butenedioic (maleic)	1286	0.041	1404	0.121	1605	0.138
trans-Butenedioic (fumaric)	1326	0.049	1378	0.108	1682	0.196
cis-Methylbutenedioic	1331	0.050	1449	0.148	1644	0.165
Methylenebutanedioic	1321	0.048	1423	0.132	1636	0.159
Tricarboxylic acids						
Propane-1,2,3-tricarboxylic	1736	0.299	1830	0.823	2143	1.569
trans-Propene-1,2,3-tricarboxylic	1754	0.323	1854	0.915	2155	1.656
1-Hydroxypropane-1,2,3-tricarboxylic	1851	0.497	1907	1.162	2184	1.880
2-Hydroxypropane-1,2,3-tricarboxylic (citric)	1847	0.488	1886	1.060	2127	1.461
Phosphoric acid	1263	0.037	1330	0.088	1683	0.197

[a] Columns, retention data and ordering of the acids as in Table I.

[b] May be obtained on acid-catalyzed lactonization (cf. Table IV).

In applying systematic nomenclature to the mono- and dicarboxylic acids, it is necessary to use a borderline between carbohydrate and organic-chemical nomenclature. The best choice was considered to be the use of carbohydrate nomenclature for acids with three or more (hydroxyl + carboxyl) groups. Accordingly, this nomenclature is used consistently in Tables I, II and IV whereas organic-chemical nomenclature is used in Table III.

Stationary Phases

The silicone stationary phases are of increasing importance and predominate in GLC work on Me_3Si derivatives at present. Because of the non-polar character of these derivatives, the best chromatographic results are usually obtained with stationary phases ranging from non-polar to medium-polar. The non-polar 100% methyl silicones and the well-defined 50% phenyl silicones are stable at high temperatures and have been used particularly extensively. Among the more polar phases, the 50% trifluoropropyl silicones have long been recognized as suited to the analysis of many classes of compounds, including Me_3Si derivatives of carbohydrates. The present investigation was confined to these three representative types of silicone stationary phases.

Similar and fairly low liquid phase loadings were used (Table I) in accordance with common practice for derivatives

Table IV. Lactones of Aldonic Acids[a]

Acid	100% Me silicone (OV-1) 160°		50% Ph, 50% Me (OV-17) 160°		50% $C_2H_4CF_3$, 50% Me (QF-1)	
	I	r	I	r	I	r
1,4-Lactones[b]						
2-Deoxytetronic	1216	0.030	1394	0.116	1867	0.57
3-Deoxytetronic	1165	0.024	1360	0.100	1695	0.31
Erythronic	1431	0.078	1585	0.271	2001	0.92
Threonic	1383	0.063	1484	0.173	1862	0.56
3,5-Dideoxy-erythro-pentonic	1165	0.024	1330	0.088	1704	0.32
3,5-Dideoxy-threo-pentonic	1183	0.026	1369	0.104	1738	0.36
3-Deoxy-2-C-(hydroxymethyl)tetronic	1390	0.065	1498	0.184	1825	0.49
2-C-Methylerythronic	1425	0.076	1519	0.202	1917	0.68
2-Deoxy-erythro-pentonic	1509	0.110	1629	0.33	2136	1.48
2-Deoxy-threo-pentonic	1536	0.124	1692	0.44	2193	1.81
3-Deoxy-erythro-pentonic	1488	0.100	1635	0.34	2019	0.98
3-Deoxy-threo-pentonic	1505	0.108	1660	0.38	2030	1.02
Ribonic	1697	0.252	1775	0.64	2230	2.06
Arabinonic	1647	0.202	1716	0.49	2059	1.13
Xylonic	1665	0.218	1737	0.54	2154	1.58
Lyxonic	1752	0.321	1832	0.83	2282	2.47
2,6-Dideoxy-ribo-hexonic	1538	0.125	1654	0.37	2149	1.55
3,6-Dideoxy-arabino-hexonic	1519	0.115	1648	0.36	2004	0.93
3-Deoxy-2-C-hydroxymethyl-erythro-pentonic	1702	0.257	1760	0.60	2097	1.29
3-Deoxy-2-C-hydroxymethyl-threo-pentonic	1715	0.272	1775	0.64	2130	1.45
2-C-Methylribonic	1657	0.211	1697	0.45	2085	1.24
2-C-Methylarabinonic	1654	0.208	1682	0.42	2027	1.01
2-Deoxy-arabino-hexonic	1813	0.42	1928	1.28	2394	3.64
2-Deoxy-lyxo-hexonic	1797	0.39	1873	1.00	2433	4.17
3-Deoxy-ribo-hexonic	1791	0.38	1855	0.92	2274	2.40
3-Deoxy-arabino-hexonic	1785	0.37	1876	1.01	2254	2.24
3-Deoxy-xylo-hexonic	1802	0.40	1900	1.13	2304	2.66
3-Deoxy-lyxo-hexonic	1808	0.41	1892	1.09	2337	2.99
6-Deoxymannonic	1791	0.38	1860	0.94	2294	2.57
6-Deoxygalactonic	1696	0.25	1745	0.56	2101	1.31
Allonic	1962	0.81	1991	1.70	2474	4.80
Altronic	1901	0.62	1912	1.19	2259	2.28
Gluconic	1932	0.71	1960	1.48	2356	3.19
Mannonic	2012	1.01	2049	2.21	2464	4.64
Gulonic	1953	0.78	1993	1.72	2389	3.58
Idonic	1956	0.79	1982	1.63	2443	4.31
Galactonic	1925	0.69	1943	1.37	2320	2.82
Talonic	1959	0.80	1991	1.70	2478	4.87
1,5-Lactones						
Xylonic[c]	1705	0.26	1785	0.67	2189	1.79
Gluconic[c]	1919	0.67	1962	1.49	2337	2.99
Mannonic	1912	0.65	1940	1.35	2363	3.27
Ascorbic acids						
erythro-Hex-2-enono-1,4-lactone	1988	0.91	2029	2.02	2439	4.25
threo-Hex-2-enono-1,4-lactone[d]	1980	0.88	2028	2.01	2469	4.72

[a] Columns, retention data and ordering of the acids as in Table I. Retention of the glucitol reference derivative on QF-1: ~ 3 min.

[b] Predominant form after acid-catalyzed lactonization.

[c] Significant proportion of the 1,5-lactone after lactonization.

[d] The L-form is vitamin C (L-ascorbic acid).

of carbohydrates and fatty acids. The specific retention volume of the non-polar Me_3Si derivatives should increase for the three silicones in the order trifluoropropyl < phenyl < methyl, i.e., in order of decreasing polarity. This was reflected in shorter retention times on the phenyl silicone column relative to the comparable methyl silicone column, and in the required use of 120°C (instead of 160°C) for the trifluoropropyl silicone. For the lactones, large contributions to the retention on the latter phase are obtained from specific polar interactions between compound and phase. As a result, 160°C was found to be appropriate for all three columns in this particular case (Table IV).

Presentation and Accuracy

Isothermal retention data for carbohydrate derivatives have been published almost exclusively as relative retentions. Evidently, there has been a reluctance to use the hydrocarbon-based Kováts' retention index, I (8), because of the lack of structural relationships between hydrocarbons and carbohydrates. However, a decisive advantage of the retention index, compared with the relative retention, is its much smaller variation with temperature (8). Differences between nominal and true column temperature often exist, particularly between different and imprecisely calibrated instruments. In the frequent applications involving comparisons with reference retention data, it is therefore preferable to use the retention index.

In Tables I-IV, both retention index and relative retentions are given. The retention index was calculated relative to the even-numbered *n*-alkanes. The Me_3Si derivative of glucitol was used as the reference in the determination of relative retention. It was normally injected to give a reference retention time both before and after duplicate runs of each derivative. The glucitol derivative was also used as a marker for determination of the retention index. The more laborious method of co-chromatography of each compound with the reference *n*-alkanes was not applied although it may give somewhat more accurate results.

The reliability in the determination of the last figure of the retention index is low for the early-eluted compounds but increases rapidly with increasing retention and is very high for the late-eluted derivatives. Conversely, the significance of the third decimal figure of the relative retention is high with the early-eluted compounds but decreases slowly with increased retention.

Correlations between Structure and Retention Index

Graphical Representation

In studies of correlations between structure and retention in GLC, the advantages of the retention index are accentuated by the fact that changes in logarithmic retention parameters may be regarded as proportional to the corresponding differences in the magnitude of the intermolecular forces between solute and stationary phase. Diagrams with retention indices on two stationary phases along the two axis are often recommended as a means for illustrating relationships between structure and retention and for extracting structural information from retention data of unknowns (9). The most fruitful results are normally obtained when one non-polar and one more polar stationary phase are used.

The present study makes use of a modified diagrammatic method in which the retention index on the polar phase is replaced by the difference, ΔI, in retention index between the polar and the non-polar phase. This concept, introduced already by Kováts (8), represents the magnitude of polar and specific interactions whereas the retention index on the non-polar phase represents the magnitude of non-polar interactions between compound and stationary phase. Since the integral retention index on the polar phase corresponds to the sum of polar and non-polar interactions, it may be argued that the I versus ΔI method gives the more direct characterization of a compound. A similar numerical approach has been used by Butts to characterize Me_3Si derivatives of many types of compounds (10).

In Figure 1, I versus ΔI diagrams are given for acyclic aldonic (Table I) and aldaric (Table II) acids. Similar diagrams

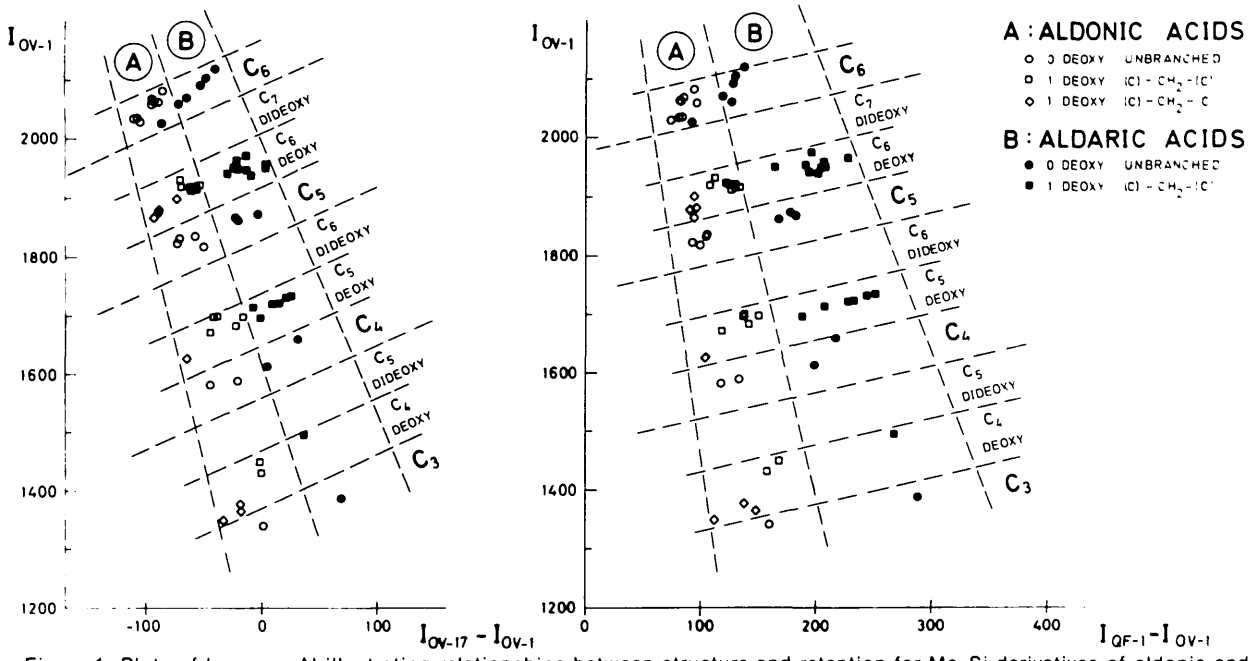

Figure 1. Plots of I versus ΔI illustrating relationships between structure and retention for Me_3Si derivatives of aldonic and aldaric acids.

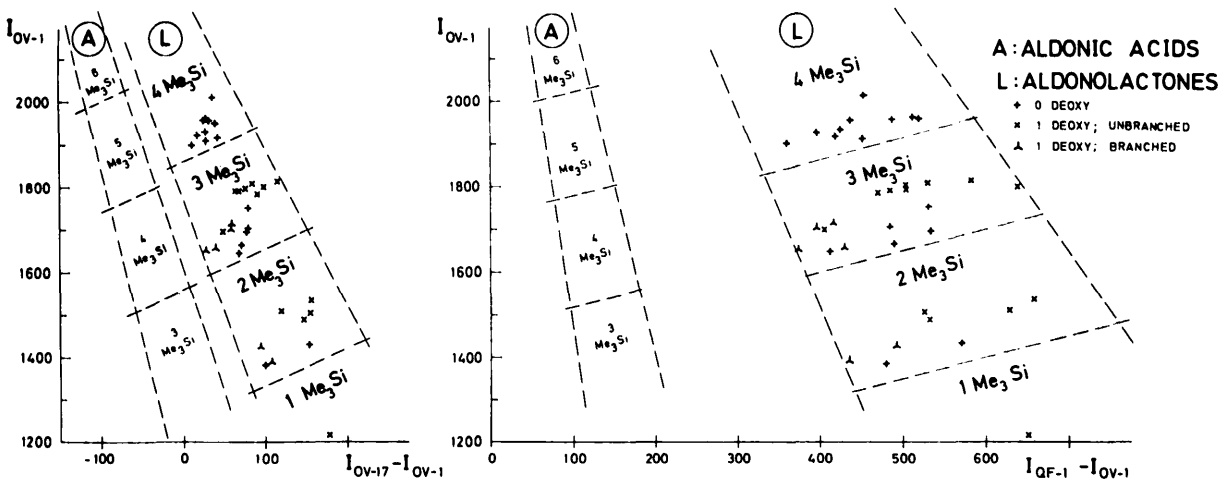

Figure 2. Plots of I versus ΔI illustrating retention characteristics of Me_3Si derivatives of aldonolactones in relation to those of the acyclic acids.

for lactones of aldonic acids (Table IV) are given in Figure 2. The position of the individual compounds have been marked only for those belonging to the indicated, frequently occurring structural groups. However, in indicating approximate borders between different structural categories, the positions of all the acyclic compounds in Tables I-II and all the lactones in Table IV have been considered.

With the aldonic and aldaric acid derivatives, the ester functional groups would be expected to give rise to the largest polar interactions. Accordingly, the aldaric acids (B) having two ester groups are located to the right of the aldonic acids (A) in the diagrammatic representation (Figure 1). Furthermore, the tricarboxylic acids appear in the region to the right of the aldaric acids, and the neutral alditols (not systematically studied) in the region to the left of the aldonic acids. The lactone group exhibits a more polar character than the silyl ester group, and the lactones (L) appear to the right of the corresponding acids (Figure 2).

Retention Index Increments

The very basis of the retention index system is the constant increment in the retention index between members of a homologous series. The addition of structural units other than the methylene group may cause similar characteristic retention index increments (11). The tabulated data and the corresponding diagrammatic representations for the investigated compounds were studied with respect to such relationships. It was found that structural units can be defined which correspond to characteristic changes in I as well as ΔI and which, furthermore, sum up to the complete structures. The approximate magnitude of the observed I and ΔI increments are given in Table V. The increments are reflected in the regular pattern of the areas for various types of structure in Figures 1-2, and in the characteristic positions of different sub-structures within these areas. The best quantitative correlations are obtained with the non-cyclic derivatives because the influence of config-

Table V. Approximate Retention Index Increments on Silicone Stationary Phases[a] Caused by Structural Units of Aldonic and Aldaric Acids

Added unit	I^{Me}	I^{Ph}	I^{F}	$I^{Ph}-I^{Me}$	$I^{F}-I^{Me}$
(C)-CH_2-(C)	100	100	120	0	20
(C)-CH_2-(H)	20	0	0	-20	-20
H-C(-OSiMe_3)-	240	200	200	-40	-40
H-C(-OCH_3)-	150	150	150	0	0
-$COOSiMe_3$ [b]	300	300	350	0	50

[a] I^{Me}: 100% Methyl silicones; I^{Ph}: 50% Phenyl, 50% methyl silicones; I^{F}: 50% Trifluoropropyl, 50% methyl silicones. [b] Replacing a hydrogen atom.

uration on retention is less prominent than with the rigid and polar lactones.

Non-polar Interactions

The non-polar (dispersion) forces between solute and stationary phase are roughly proportional to the external surface area of the solute molecule. Since the retention index on the methyl silicone reflects the magnitude of these forces, the increments given in the first column of Table V can be interpreted in terms of surface area increments caused by the structural units. A CH_2 group inserted in a carbon chain lengthens the molecule and exerts an effect similar to that in a typical homologous series. In contrast, a CH_2 group added as a methyl group is essentially masked under the large Me_3Si umbrellas and contributes very little to an increased outer surface. The increments from silyl ether and ester groups are very small in relation to their content of atoms because of their compact sphere-like structure. Similar reasoning can explain more subtle effects of structure which determine the exact retention index. In detailed interpretations, it should be observed that small polar contributions which differ for solutes of varying polarity can be attributed to the rudimentary polar character of the methyl silicone.

Non-polar interactions are also responsible for the major contributions to the retention of the investigated derivatives on the phenyl and trifluoropropyl silicones. This is demonstrated by the low $\Delta I/I$ ratios. However, the $\Delta I/I$ ratio cannot be used as an absolute measure of the magnitude of polar interactions relative to non-polar interactions. This may be ascribed to a difference in the ratio between non-polar forces for solutes in general and for *n*-alkanes on different phases. The negative rather than positive ΔI values with the phenyl silicone are illustrative. Compared to the flexible *n*-alkanes, the bulky Me_3Si derivatives develop less intimate contact with the phenyl silicone, containing large and planar phenyl groups, than with the methyl silicone. As a result, the dispersion forces contribute less to the retention index for the phenyl than for the methyl silicone, and anomalously low ΔI values are obtained. A similar effect on individual ΔI values on one particular phase may be caused by the structure of the solute. A bulky and inflexible structure decreases ΔI for silicones having large and inflexible substituents such as the phenyl group.

Polar Interactions

The polar contribution to the retention of a compound depends not only on the number and type of functional groups but also on the availability of these groups for short-distance interactions with the phase. The magnitude of dipole interactions decreases extremely rapidly with the distance between the charges. A characteristic feature of the Me_3Si derivatives is that the polar functional groups are located in the interior of the molecule whereas the outer surface is non-polar because of the abundant silicone-linked methyl groups. A well-known first consequence of this structural feature is the non-polar character of the derivatives. A less often recognized but important consequence is that the polar contribution to the retention is governed by the molecular geometry to a much larger extent than with most other compounds. Significant polar contributions are obtained only if the constitution and configuration of the derivative permit intimate contact between its functional groups and the stationary phase.

The related principles permit an interpretation of the approximate ΔI ($I^{Ph} - I^{Me}$ and $I^{F} - I^{Me}$) increments given in Table V for the structural elements of the acyclic aldonic and aldaric acids. The addition of a (C)-CH_2-(C) group makes the derivative less compact and may increase the availability of the polar ester functional groups, particularly for interaction with the trifluoropropyl silicone. Conversely, a (C)-CH_2-(H) group makes the derivative more compact and strengthens the non-polar character of the derivative significantly. The screening effect may be ascribed to the methyl group either directly, as with the 2-*C*-methyl-substituted acids, or indirectly through steric interactions with $OSiMe_3$ groups. Similar effects of methyl groups on retention have been observed with alkyl esters of branched, unsubstituted acids (12). The insertion of a $HCOSiMe_3$ unit into the structure causes a strongly increased screening of the polar groups. The decrease in ΔI with increasing molecular weight for compounds belonging to the same structural class (Figures 1-2) is explained mainly by this effect. However, as indicated above, non-polar effects may contribute to the decrease in ΔI, at least with the phenyl silicone. Polar interactions between the silyl ether group and the stationary phase appear to be almost completely prevented by the Me_3Si umbrella. With the $HCOCH_3$ unit, the polar contribution from the methyl ether group roughly compensates for the screening effect on other polar groups. The $COOSiMe_3$ group would be expected to cause a large increase in ΔI with regard to the polar character of an ester group. Counteracting effects, similar to those for the $HCOSiMe_3$ unit, compensate for (phenyl silicone) or reduce (trifluoropropyl silicone) this potential polar effect. The figures given in Table V demonstrate that the reduced availability of the functional groups causes large effects in the polar properties of the Me_3Si derivatives which would be unpredictable from considerations of the functional groups alone.

Within the structural categories discussed, correlations between constitutional isomerism and ΔI increments can be found. Thus the effect of branching is similar to the effect of addition of a (C)-CH_2-(H) group for most of the aldonic and aldaric acids. Among diastereomers, the configurations of the individual derivatives determine the preferred conformations and consequently influence the accessibility of the polar groups. As previously discussed (2), a favoured antiparallel conformation (normally induced by an *erythro* configuration) of the $OSiMe_3$ groups linked to the α- and β-carbon atoms gives rise to the most effective screening of the ester group. Consequently, the lowest ΔI values are associated with this conformation.

It is emphasized that Table V and Figures 1-2 illustrate an average behaviour of the type of derivatives investigated. Several deviations exist, but apparently anomalous behaviour can often be explained by unusual structural features as exemplified by *C*-methylglyceraric acid and mannaric acid. The low ΔI values of *C*-methylglyceraric acid compared with glyceraric acid can be ascribed to a screening effect of the methyl group on both ester groups. Mannaric acid falls into the aldonic acid region because its configuration favours antiparallel conformation of the α-and β-substituents at both ester groups and renders the structure strongly non-polar. Since Figures 1-2 indicate the position of those structural categories which are best represented, certain exceptional compounds may fall outside the indicated areas in spite of normal behaviour. As an example, 3,4-dideoxyhexonic acid falls into the aldaric acid region because of the two (C)-CH_2-(C) groups.

With the lactones (Figure 2), the effects on ΔI of various groups are qualitatively similar to those observed with the acyclic derivatives. As anticipated, the screening effects tend to be larger because of the high polarity of the lactone group. Quantitative estimations cannot easily be made because of the large influence of configuration. As previously noted (5),

striking configurational effects can be related to the positions of the substituents of the lactone ring. Location of these substituents, particularly those at C-2 and C-3, on the same side of the ring increases retention. Obviously, this is explained by the increased accessibility of the lactone group for interaction with the stationary phase.

Qualitative Analytical Information

The qualitative analytical information available from the retention data may be divided into three categories.

The traditional application is the identification of compounds for which reference data are available. Retention index is preferable to relative retention for this purpose, and analysis on two stationary phases permits a much greater certainty than analysis on one phase. The I versus ΔI diagram indicates the type of alternative compounds which may have similar retention data.

A second type of information can be obtained without access to retention data for the compound of interest. In this case, the increased information provided by analysis on two phases and the use of a diagrammatic representation is almost a prerequisite. The position of the unknown in the I versus ΔI diagram indicates the type of structure and permits efficient exclusion of structural possibilities. Comparison with the position of known compounds in the same region in terms of I and ΔI increments may indicate the exact structure.

Thirdly, the approximate retention index data for a given compound can be calculated. One or two known compounds are chosen, the structure of which can be converted to the one of interest by the addition of structural units with a predictable effect on retention (cf. Table V). The corresponding I and ΔI increments (Table V) are added to the I and ΔI values of the reference coumpounds. The increments may be modified towards the values observed with closely related structural changes. Approximate calculation of retention data is of particular value when the possible presence or the absence of one or a few inaccessible compounds is to be demonstrated. Such applications occur frequently, i.e., when some information from chemical or from other analytical methods is available.

Retention Data in Temperature-Programmed Analysis

The development of adequate instrumentation and stationary phases has brought about an increased use of temperature programming in gas chromatography. The wider range of compounds which can be covered in each run makes this technique attractive for the analysis of the hydroxy acid derivatives. A crucial problem in changing method is to find a system for retention data, capable of giving as much information as that for isothermal data discussed above.

The MU Concept

The methylene unit (MU) system (13) appears to be the most versatile measure of retention in linear temperature-programmed analysis, and its application to the compounds studied was therefore investigated. The MU system is based on the almost linear relationship between the retention time and the number of methylene units of *n*-alkanes. This relationship is illustrated in Figure 3 for three columns used for programmed analysis. The MU value of a compound is determined by linear interpolation between the *n*-alkanes eluting before and after the compound. Since the I values are obtained by a corresponding logarithmic interpolation, the approximate relationship MU ≈ 0.01×I is obtained. Therefore the same type

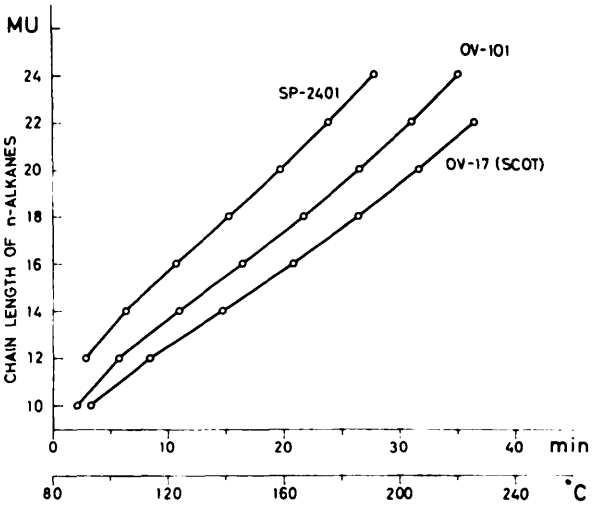

Figure 3. Retention data of *n*-alkanes for determination of MU in temperature-programmed analysis (cf. Table VI).

of qualitative information can be obtained by the use of the MU concept in programmed analysis as by the previously described use of the retention index in isothermal analysis.

The equipment for temperature programming of modern instruments offers high accuracy, and highly reproducible MU values can normally be obtained under unchanged analytical conditions. The essential question in applying the MU concept is therefore to what extent different experimental conditions and different columns and stationary phases influence the result. A comparison of MU values, obtained under conditions suited for programmed analyses, with the isothermal retention index values was therefore made for representative derivatives (Table VI). The low-viscosity OV-101 and SP-2401 phases employed offer good chromatographic results for early-eluted compounds. It is seen that very small differences between MU and 0.01×I are obtained with the methyl and phenyl silicones, although both phase and support are different for the methyl silicone, and although a completely different capillary (SCOT) column was used for the phenyl silicone. Published (14) MU values for some aldonic acid and aldonolactone derivatives on a wall-coated open tubular (WCOT) column coated with SE-30 also differ very little from the methyl silicone data of Table VI. The larger differences observed with the trifluoropropyl silicones were shown to exist between retention indices on the two columns as well, and consequently they are due primarily to the different packings. The deviations are largest with the most polar derivatives, but their approximate magnitude can be predicted empirically from the tabulated data. Actually, the SP-2401 phase would be expected to differ from the old QF-1 phase, e.g., in molecular uniformity, and a difference is also demonstrated by different McReynolds' numbers. In conclusion, the results demonstrate that the use of the I and MU concepts permits useful interrelation of isothermal and temperature-programmed data for a particular type of phase even if widely different analytical conditions are used.

Influence of Temperature

The retention index of a compound is a function of temperature although it varies far less than the relative retention. This function is of interest when MU values are used in temperature-programmed analysis because the MU values may be

Table VI. Comparative Retention Data from Temperature-Programmed Analysis[a]

	100% Me silicone OV-101 [b]		50% Ph, 50% Me OV-17 (SCOT) [c]		50% $C_2H_4CF_3$, 50% Me SP-2401 [d]	
	MU[e]	MU-0.01·I [f]	MU[e]	MU-0.01·I [f]	MU[e]	MU-0.01×I [f]
Aldonic acids (cf. Table I)						
Glyceronic (glyceric)	13.43	0.03	13.56	0.15	14.81	-0.19
2-C-Methylglyceronic	13.33	-0.15	13.18	0.04	14.31	-0.29
2-Deoxytetronic	14.52	0.04	14.60	0.14	15.93	-0.23
Xylonic	18.11	-0.07	17.67	0.00	19.11	-0.06
3-Deoxy-2-C-hydroxymethyl-erythro-pentonic	19.29	-0.02	18.62	0.02	20.33	-0.10
Mannonic	20.33	-0.01	19.26	0.03	21.23	0.08
Aldaric acids (cf. Table II)						
Glyceraric (tartronic)	14.00	0.14	14.63	0.08	16.40	-0.35
Deoxytetraric (malic)	15.08	0.14	15.40	0.10	17.30	-0.32
Erythraric	16.14	0.01	16.19	0.02	17.92	-0.20
3-Deoxy-2-C-hydroxymethyl-threo-pentaric	19.34	-0.06	19.02	-0.08	21.09	-0.25
Glucaric	20.68	-0.02	20.04	-0.01	21.87	-0.02
Non-carbohydrate acids (cf. Table III)						
Shikimic	18.39	-0.05	18.63	-0.08	20.22	-0.31
Butanedioic (succinic)	13.07	0.16	13.91	0.11	15.68	-0.48
Hexanedioic (adipic)	15.06	0.16	15.92	0.06	18.01	-0.48
cis-Butenedioic (maleic)	12.94	0.08	14.06	0.02	15.59	-0.46
Lactones (cf. Table IV)[g]						
Arabinonic	16.45	-0.02	17.05	-0.11	20.02	-0.57
3-Deoxy-arabino-hexonic	17.84	-0.01	18.67	-0.09	22.04	-0.50
Gluconic	19.30	-0.02	19.49	-0.11	23.07	-0.49
Ascorbic	19.77	-0.03	20.17	-0.11	24.07	-0.62

[a] Initial temperature: 80°. Programming: 4°/min from start to 240°.

[b] Column: 2 m · 0.2 cm i.d. Packing: 3% OV-101 on 100/120 mesh Gas-Chrom Q. Carrier gas: ~30 ml/min purified nitrogen.

[c] Column: 15 m · 0.02 cm o.d. stainless steel support-coated open tubular (SCOT) column loaded with OV-17 (Perkin-Elmer Co.). Carrier gas: ~5 ml/min pressure-regulated (13 psig) helium.

[d] Column: 3 m · 0.2 cm i.d. Packing: 3% SP-2401 on 100/120 mesh Supelcoport. Carrier gas: ~20 ml/min purified nitrogen.

[e] Determined by linear interpolation between the even-numbered n-alkanes (cf. Figure 3).

[f] Retention indices from Tables I-IV.

[g] 1,4-Lactones.

regarded as representing retention index values at varying temperatures. Actually, changes of stationary phase loading, column length, carrier gas flow, and program rate may also be regarded as equivalent to changes in temperature with respect to their influence on MU.

An interpretation of the difference (MU - 0.01×I) in terms of the influence of temperature requires a relation between MU and the corresponding "averaged" column temperature. In the present study such a relation is obtained from Figure 3 and from the estimation that the elution temperature is 10-15° higher than the average temperature. In combination with the difference between the average temperature and the temperature used for determination of the retention index, the basic dependence of the retention index on temperature should determine (MU - 0.01×I) for a given column. The change in the retention index with temperature may often be predicted from the structure of a compound on thermodynamic grounds (9). Thus, increased branching normally leads to a larger increase (or a smaller decrease) in the retention index with increasing temperature. A striking example is the relation between glyceronic acid and the branched non-polar *C*-methylglyceronic acid which are eluted at low temperatures. The branched acid exhibits lower (MU - 0.01×I) values as predicted, and on the methyl silicones a reversed elution order on programming compared to isothermal analysis at 120° is observed. It is concluded that the influence of temperature is the crucial factor in interpretations and predictions of differences between 0.01×I and MU and between MU values obtained under different experimental conditions.

Analytical Applications

Temperature-programmed analysis under standardized conditions (cf. Table VI) is now used in this laboratory for routine

analysis of samples containing the types of compounds discussed. Each sample is analysed simultaneously on two parallel columns (methyl + phenyl silicone or methyl + trifluoropropyl silicone). An internal polyol standard is used as a marker in the calculation of MU values. These MU values are used for qualitative analysis as described above for the retention index. Anticipated deviations (MU - 0.01×I) are considered when required, particularly with the phenyl and trifluoropropyl silicones. Compared to isothermal analysis, the disadvantage of small deviations from the reference data is more than compensated by the wider range of compounds (including monohydroxy monocarboxylic acids) which can be analysed simultaneously.

An important question in the use of temperature programming is whether isothermal (I) or programmed (MU) reference data are to be preferred. The independence of most experimental conditions except temperature is an argument for isothermal data. On the other hand MU values can be determined with an almost invariable accuracy for a much wider range of compounds. The deviations between MU values obtained under different conditions are also likely to be smaller and more uniform than deviations between I and MU values. Furthermore, MU values are more easily obtained because no change of the conditions used for analytical applications is required. When the reference data are intended primarily for temperature-programmed work, the use of MU values therefore appears preferable.

Acknowledgments

The author thanks Lena Månbladh, Åke Andersson and particularly Mary Mattsson for skillful experimental assistance. The financial support of Carl Tryggers Stiftelse för Vetenskaplig Forskning is gratefully acknowledged.

Manuscript received March 10, 1977;
revised manuscript received May 31, 1977.

References

1. C.C. Sweeley, R. Bentley, M. Makita, and W.W. Wells. Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances. *J. Amer. Chem. Soc.* **85:** 2497-2507 (1963).
2. G. Petersson. Gas-chromatographic analysis of sugars and related hydroxy acids as acyclic oxime and ester trimethylsilyl derivatives. *Carbohyd. Res.* **33:** 47-61 (1974).
3. I. Matsunaga, T. Imanari, and Z. Tamura. Simultaneous determination of urinary uronic acids and saccharic acids by gas chromatography. *Chem. Pharm. Bull.* **18:** 2535-43 (1970).
4. L. Jansén and O. Samuelson. Separation of dicarboxylic hydroxy acids by anion-exchange chromatography and gas chromatography. *J. Chromatog.* **57:** 353-64 (1971).
5. G. Petersson, H. Riedl, and O. Samuelson. Gas chromatographic separation of aldonic acids as trimethylsilyl derivatives. *Sv. Papperstidn.* **70:** 371-75 (1967).
6. M. Matsui, M. Okada, T. Imanari, and Z Tamura. Gas chromatography of trifluoroacetyl derivatives of alditols and trimethylsilyl derivatives of aldonolactones. *Chem. Pharm. Bull.* **16:** 1383-87 (1968).
7. I.M. Morrison and M.B. Perry. The analysis of neutral glycoses in biological materials by gas-liquid partition chromatography. *Can. J. Biochem.* **44:** 1115-26 (1966).
8. E. Kováts. Gas-chromatographische Charakterisierung organischer Verbindungen. *Helv. Chim. Acta* **41:** 1915-32 (1958).
9. A.B. Littlewood. *Gas Chromatography,* Academic Press, 1970, pp. 107-118.
10. W.C. Butts. Two column gas chromatography of trimethylsilyl derivatives of biochemically significant compounds. *Anal. Biochem.* **46:** 187-99 (1972).
11. G. Schomburg and G. Dielmann. Identification by means of retention parameters. *J. Chromatog. Sci.* **11:** 151-59 (1973).
12. J.K. Haken, D.K.M. Ho, and M. Wainwright. Gas chromatography of homologous esters. VIII. Reduced retention of *n*- and isoalkyl pivalate esters. *J. Chromatog.* **106:** 327-33 (1975).
13. C.E. Dalgliesh, E.C. Horning, M.G. Horning, K.L. Knox, and K. Yarger. A gas-liquid-chromatographic procedure for separating a wide range of metabolites occurring in urine or tissue extracts. *Biochem. J.* **101:** 792-810 (1966).
14. J. Szafranek, C.D. Pfaffenberger, and E.C. Horning. Separation of aldonic, deoxyaldonic, hexuronic and hexaric lactones and acids using thermostable open tubular glass capillary columns. *J. Chromatog.* **88:** 149-56 (1974).