Ammonia-Isobutane Chemical Ionization Mass Spectra of Oligosaccharide Peracetates[1,2]

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Chemical ionization mass spectra with ammonia and isobutane as the reagent gas are reported for the peracetates of ten disaccharides, six trisaccharides, four tetrasaccharides, and two pentasaccharides. Intense ions which corresponded to attachment of an ammonium ion to the molecule were observed for all but the pentasaccharide acetates. Ions which corresponded to ammonium ion attachment to thermolysis fragments generally dominated the fragmentation patterns. The relative importance of thermolytic fragments increased with increasing molecular weight. Ammonium ion attachment spectra can be used to detect reliably molecular weights for oligosaccharide acetates through tetrasaccharides. Thermal fragment ammonium ion complexes permit the unequivocal establishment of the masses of subunits within the parent sugar, and the glycosyl fragment ions in the spectra give unequivocal information about the nature of the nonreducing end of the oligosaccharide chain. The spectra appear to be quite sensitive to the source conditions because of the thermolysis reactions involved. Nonetheless, ammonia chemical ionization should be a truly complementary technique to electron impact mass spectra for oligosaccharide structure determination.

The use of mass spectrometry for sequencing oligosaccharides is considerably more complex than the corresponding problem for polypeptides. The increased difficulty is due to the fact that structural variations in carbohydrates are generally much more subtle than structural differences in peptides, *i.e.*, the position and stereochemical isomerization in carbohydrates is much more subtle than the differences between amino acid subunits in peptides. Furthermore, the molecular ions of carbohydrate derivatives are generally unstable. In spite of these difficulties, electron impact mass spectra have been successfully used for structure elucidation of carbohydrate derivatives.[3-11] The most commonly used derivatives are acetates,[5,6] methyl ethers,[7,8] and trimethylsilyl ethers.[6,9-11] Electron impact mass spectra of all of these derivatives fail to show molecule ions, and high molecular weight fragments characteristically have very low intensities.

Two approaches have been used to overcome the low intensity of high mass ions in carbohydrate mass spectra. Williams and Yeo[12] proposed derivatization by a functional group with a low ionization potential to reduce fragmentation of the backbone. This approach has been used in recent studies of oligosaccharide mass spectra by Cooks, *et al.*,[13] and Chizhov, *et al.*[14]

The requirement of an extra derivatization step makes these methods unattractive; the problem is especially acute when small amounts of samples are involved.

The second approach to obtaining molecule ion information from carbohydrate mass spectra involves use of "gentle" methods for ionization, *e.g.*, field ionization (FI), field desorption (FD), and chemical ionization (CI). FI and FD mass spectra of a few oligosaccharide derivatives have been reported by Krone and Beckey.[15] In contrast to EI mass spectra, FD mass spectra generally show so little fragmentation that their utility in structural investigations is limited.

Hogg and Nagabhushan[16] have reported the CI mass spectra of several monosaccharides and their peracetate derivatives using methane or ammonia as reagent gases. In general, the ammonia CI spectra were dominated by ammonium ion complexes (AIC) with the molecule, while the methane CI spectra gave characteristic fragment ions which gave information about groups attached to the pyranose ring.

In our experience the CI mass spectra of oligosaccharide peracetates or pertrimethylsilyl ethers, using methane or isobutane as the reagent gas, are very similar to the corresponding EI mass spectra. That is, the spectra were more or less dominated by ions resulting from cleavage of the nonreducing terminal glycosidic linkage, and the intensities of ions with high masses were characteristically low. During these experiments we found that mixtures of ammonia and isobutane[17] (2:1) at roughly one-third Torr (40 pascals) gave the best results. In this report we will discuss the ammonia CI mass spectra of 22 oligosaccharide peracetates. The numerical designations, names, structures, and molecular weights of the peracetates for these oligosaccharides are listed in Table I.

Ammonia-Isobutane CI Mass Spectra of Disaccharide Peracetates. Table II lists the ten most intense ions in the ammonia-isobutane CI mass spectra of the disaccharide acetates listed in Table I. The listing has been abbreviated to the ten most intense ions because of the sensitivity of the spectra to source parameters and the general observation that abbreviated listings of spectra are generally sufficient for analytical purposes. Small changes in source conditions, particularly temperature, generally caused extensive changes in the low-intensity ions observed in the spectra; since these ions were generally less than 1% of the base peak, they have been neglected in the listing. In cases where low-intensity ions are of particular chemical or structural interest they will be mentioned in the text along with their relative intensity.

In all cases the disaccharide acetate ammonium ion complex (AIC) was the base peak in the spectrum (m/e 696 for 1-8 and m/e 624 for 9 and 10) above the reagent gas region (m/e >60). The spectra also generally contained low-intensity cluster ions above the molecule AIC. The mass spectra of 1 and 3 contained small peaks at m/e 798 which corresponded to $[M + NH_4 + Ac_2O]^+$ ions. This ion decomposed with loss of ammonium acetate to give a $[M + Ac]^+$ ion at m/e 721. A metastable ion was observed

Table I
Names, Structures, and Molecular Weights of Peracetyl Derivatives of Oligosaccharides Examined in This Study

Compd	Name	Structure	Mol wt of peracetate derivative
Disaccharides			
1	Mannobiose	α-D-Man*p*1→2-D-Man*p*	678
2	Sophorose	β-D-Glc*p*1→2-D-Glc*p*	678
3	Laminaribiose	β-D-Glc*p*1→3-D-Glc*p*	678
4	Maltose	α-D-Glc*p*1→4-D-Glc*p*	678
5	Cellobiose	β-D-Glc*p*1→4-D-Glc*p*	678
6	Melibiose	α-D-Glcal*p*1→6-D-Glc*p*	678
7	Gentiobiose	β-D-Glc*p*1→6-D-Glc*p*	678
8	Sucrose	α-D-Glc*p*1→2-D-Fru*f*	678
9	3-*O*-α-D-Glucopyranosyl-D-arabinopyranose	α-D-Glc*p*1→3-D-Ar*p*	606
10	6-*O*-α-L-Arabinopyranosyl-D-glucopyranose	α-L-Ar*p*1→6-D-G*p*	606
Trisaccharides			
11	Mannotriose	α-D-Man*p*1→(2-α-D-Man*p*1)$_2$	966
12	Laminaritriose	β-D-Glc*p*1→(3-β-D-Glc*p*1)$_2$	966
13	1-Kestose	β-D-Fru*f*2→1-β-D-Fru*f*2→1-α-D-Glc*p*	966
14	Planteose	α-D-Glcal*p*1→6-β-D-Fru*f*2→1-α-D-Glc*p*	966
15	Raffinose	α-D-Glcal*p*1→6-α-D-G*p*1→2-β-D-Fru*f*	966
16	Cellotriitol	β-D-Glc*p*1→4-β-D-Glc*p*1→4-Glc-OH	1010
Tetrasaccharides			
17	Mannotetraose	α-D-Man*p*1→(2-α-D-Man*p*1)$_3$	1254
18	Laminaritetraose	β-D-Glc*p*1→(3-β-D-Glc*p*1)$_3$	1254
19	Stachyose	(α-D-Glcal*p*1→6)$_2$-α-D-Glc*p*1→2-β-D-Fru*f*	1254
20	Nystose	(β-D-Fru*f*2→1)$_2$-α-D-Glc*p*	1254
Pentasaccharides			
21	Mannopentaose	α-D-Man*p*1→(2-α-D-Man*p*1)$_4$	1542
22	Laminaripentaose	β-D-Glc*p*1→(3-β-D-Glc*p*1)$_4$	1542

Table II
Monoisotopic Ammonia–Isobutane CI Mass Spectra of Peracetylated Disaccharides[a]

Compd	Peracetylated sugar											
1	Mannobiose	*m/e*	696	619	433	408	331	246	186	181	169	109
		rel intensity	100	40	2.5	5.7	26	3	2.1	4.7	5	3
2	Sophorose	*m/e*	696	654	638	636	408	366	364	331	120	102
		rel intensity	100	4	1.6	1.9	5	3.7	1.3	1.5	1.4	1.5
3	Laminaribiose	*m/e*	696	408	366	348	331	306	303	289	246	186
		rel intensity	100	7.6	36	7.1	4.1	8.7	8.2	2.0	9.5	3.8
4	Maltose	*m/e*	696	636	619	516	408	366	331	303	220	186
		rel intensity	100	2.6	35	1.8	1.7	1.3	3.0	4	2.5	1.7
5	Cellobiose	*m/e*	696	654	636	618	578	366	331	246	186	119
		rel intensity	100	2.0	1.6	2.8	1.8	1.2	1.5	2.1	2.9	1.5
6	Melibiose	*m/e*	696	447	408	366	331	303	220	186	134	118
		rel intensity	100	1.1	101	1.3	11	4.	5.5	1.3	1.0	1.5
7	Gentiobiose	*m/e*	696	654	636	576	408	394	366	331	306	186
		rel intensity	100	2.5	1.	1.5	1.	1.2	1.3	5.	1.	2.2
8	Sucrose	*m/e*	696	452	391	331	313	180	170	163	131	114
		rel intensity	100	6	22	5.7	9	20.6	100	7.8	6.5	16
9	3-*O*-α-D-Glucopyranosyl-D-arabinopyranose	*m/e*	624	564	517	506	331	278	199	186	141	139
		rel intensity	100	5.8	30	2.3	1.8	1.	1.5	1.1	1.	1.2
10	6-*O*-α-D-Arabinopyranosyl-D-glucopyranose	*m/e*	624	564	547	506	408	308	294	259	180	170
		rel intensity	100	1.8	2	5.8	1.1	1.4	1.4	7.3	1.7	2.8

[a] Only the ten most intense ions below *m/e* 696 are reported.

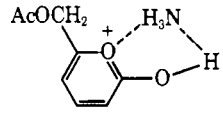
for this transition in the spectrum of 1. Cluster ions are a normal feature of CI mass spectra, and, as their intensities are subject to substantial variations depending on the source conditions, they have not been included in the tabulations.

The most striking feature of the spectra in Table II is the abundance of even-electron ions with even mass. More than two-thirds of the major ions in these CI spectra had even masses. Since virtually all of the ions obtained under these conditions are even-electron ions,[17] the even-mass ions must contain a nitrogen atom. There are at least four ways in which nitrogen-containing fragment ions could appear in these spectra, and at least three of them appear to be important. The first is, of course, direct CI fragmentation of a molecular AIC with loss of a small molecule. The spectrum of peracetylmaltose (4) shows an energy-broadened metastable ion at *m/e* 581.2 which corresponds to the transition *m/e* 696 → *m/e* 636, *i.e.*, loss of acetic acid from the molecular AIC. Similar low-intensity metastable ions were observed in the spectra where *m/e* 636 was a prominent ion.

The second mechanism for formation of fragment AIC's involves thermolysis of the parent molecule followed by NH_4^+ attachment to the neutral fragments. This is the most likely process for formation of the ions at *m/e* 408 which correspond to peracetylated hexopyranose AIC's. The other neutral fragment from this thermolysis reaction would be a hexopyranose acetate less the elements of acetic anhydride. AIC's for these neutral fragments were prominent at *m/e* 306 in the spectra of 3 and 7, and they were present as low-intensity ions in virtually all of the other disaccharide spectra. The detailed spectra of 1 and

10 suggested that the reducing end of the chain is more likely to become the peracetylated sugar fragment in thermolysis reactions by about a factor of 10. The $[M - CH_2CO + NH_4]^+$ ions, m/e 654 in the spectra of **2**, **5**, and **7**, probably arise by pyrolytic ketene elimination followed by ammonium ion attachment, as there were no metastable ions which corresponded to loss of 42 daltons from the molecular AIC.

Even-electron nitrogen-containing ions can also be formed by attachment of neutral ammonia to CI fragment ions in the source. The ion **23**, m/e 186, which was prominent in the spectra of seven out of ten disaccharide acetates, was most probably formed in this way. We were unable to observe a metastable ion for formation of m/e 186 even under defocusing conditions. On the other hand, virtually all of the spectra showed metastable ions for formation of the ion at m/e 169, which would be a precursor to **23** by attachment of ammonia. The ion at m/e 348 in the spectrum of peracetyllaminaribiose was probably formed by an analogous attachment of ammonia to the glucosyl cation, m/e 331.

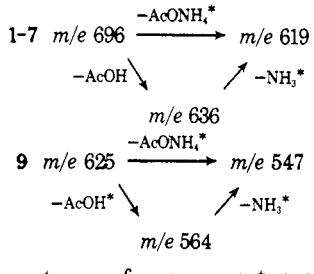


23, m/e 186

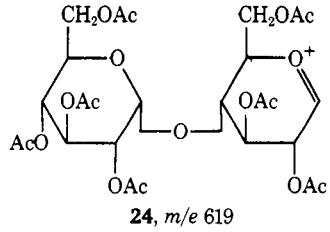
The last of the likely processes for forming nitrogen-containing ions in this CI system is chemical condensation of the saccharide acetates with ammonia followed by ionization. We were unable to find evidence of this in any of the spectra; however, this process has been observed in the ammonia CI spectra of aldehydes and ketones.[18]

Fragment ions which corresponded to the elimination of acetic acid or ammonium acetates from the molecular AIC were observed in all of the spectra and were prominent in more than half of the spectra. Metastable ions in the spectra of **1**, **3**, **5**, and **9** indicated that the elimination of ammonium acetate could occur in either one or two steps (Scheme I).

Scheme I

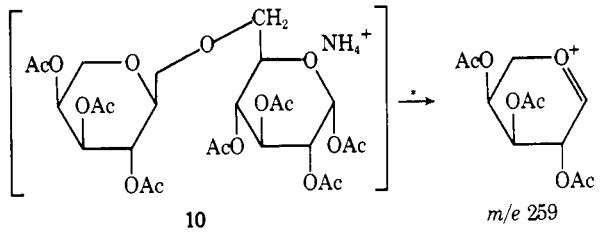


Since the spectrum of sucrose octaacetate had no ion current at m/e 619, it can be assumed that the C-1 acetoxy group is cleaved in this reaction. Thus for peracetylmaltose, for example, the m/e 619 ion would be **24**.

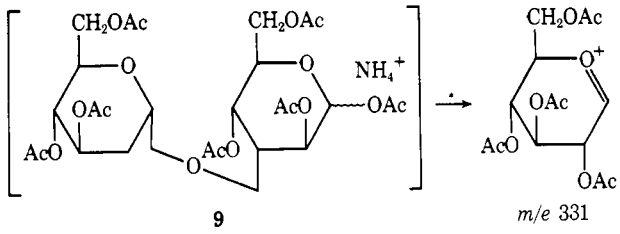


24, m/e 619

Cleavage of the glycosidic linkage results in the formation of two series of even-electron, odd-mass ions. The parent ion of the more abundant series is the glycosyl ion, m/e 331 for **1-9** and m/e 259 for **10**. Metastable transitions indicate that either the molecular AIC or the m/e 619 ion, *e.g.*, **23**, can be the precursor of this ion. The rate constant for formation of the glycosyl ion from the nonreducing terminus must be approximately 100 times that for the reducing end as judged by the relative peak heights for m/e 331 and 259 in the spectra of **9** and **10**. The alternate cleavage (formation of m/e 331 by **10** and m/e 259 by **9**) essentially does not occur under these CI conditions, although this reaction is well shown in EI mass spectra of sugar acetates.[3]



10 m/e 259



9 m/e 331

Further reactions of the glucosyl ion appear to proceed in a manner precisely analogous to the EI mass spectra[19] of the disaccharide acetates. Sequential elimination of acetic acid and ketene units, often with corresponding metastable ions, accounts for the odd-mass ions between m/e 331 and 109.

Ammonia-Isobutane CI Mass Spectra of Tri-, Tetra-, and Pentasaccharide Peracetates. The ammonia-isobutane CI mass spectra of the peracetates of tri-, tetra-, and pentasaccharides followed the patterns established by the disaccharide acetates. Table III lists the 15 most intense ions in the ammonia CI mass spectra of the oligosaccharide acetates examined in this study. The spectra are by and large dominated by nitrogen-containing ions, and the three mechanisms for formation of these ions that were identified in the disaccharide mass spectra appear to operate here as well. Decomposition of the molecular AIC, with formation of glycosyl ions consisting of a monosaccharide unit (or units) from the nonreducing end of the chain, was responsible for most of the nitrogen-free ions in the spectra. Prominent thermolysis reactions included elimination of ketene or acetic acid and cleavage of glycosidic linkages with the formation of full or partial acetates of the saccharide molecular fragments.

The relative importance of the several possible reaction pathways depends on the structure of the oligosaccharide. Thermolysis reactions tend to become more important with increasing molecular weight. For example, with the exception of laminaritriose (**12**), the base peak in the ammonia CI spectra of the trisaccharides **11-16** was the molecular AIC (m/e 984 for **11-15** and m/e 1028 for **16**). In general, the laminaridextrin peracetates **3**, **12**, **18**, and **22** have shown low thermal stability in comparison to the other oligosaccharide peracetates. Adduct ions with masses greater than the molecular AIC occurred in all of the spectra in which intense molecular AIC's were observed. These ions were generally of low intensity, although decreasing the source temperature always increased their prominence.

Decomposition of the molecular AIC through cleavage of glycosidic bonds leads to tri-, di- and monoglycosyl ions at m/e 907, 619, and 331, respectively. Direct formation of these ions from the molecular AIC was supported by appropriate metastable transitions in the mass spectra of **11**

Table II: Monoisotopic Ammonia–Isobutane CI Mass Spectra of Peracetylated Tri-, Tetra-, and Pentasaccharides[a]

Tri-saccharides	11	Mannotriose	*m/e*	984	942	924	798	696	654	636	408	366	331	264	246	204	187	134
			rel intensity	100	2.5	2.3	13.5	31	5	3.7	11	6.7	5.2	28	9.6	5.5	1.8	5.5
	12	Laminaritriose	*m/e*	984	798	696	636	619	408	366	348	331	303	289	264	246	220	186
			rel intensity	1.5	100	42	3.7	1.8	20	45	29	5.6	7.6	3.6	3.5	20	7.0	10
	13	Kestose	*m/e*	984	942	696	654	594	366	348	331	306	289	246	192	186	168	115
			rel intensity	100	1.5	6.7	2.4	1.8	3.1	2.4	6.8	5.5	2.	3.7	2.5	10.5	1.5	1.6
	14	Planteose	*m/e*	984	942	882	696	654	636	619	594	480	474	366	334	331	306	246
			rel intensity	100	1.	0.2	0.2	0.4	0.3	1.1	0.4	0.4	0.5	1.4	0.4	1.6	1.4	1.1
	15	Raffinose	*m/e*	984	942	696	682	654	594	534	492	480	474	408	366	331	289	153
			rel intensity	100	1.	0.4	0.3	0.8	0.4	0.5	0.2	0.2	0.3	0.2	1.0	9.1	1.0	1.3
	16	Cellotriitol	*m/e*	1028	986	740	698	696	624	452	410	366	364	334	276	167	153	151
			rel intensity	100	2.2	10.2	1.8	1.	3.	1.	2.3	3	5	3.2	4.8	32	3.4	20
Tetra-saccharides	17	Mannotetraose	*m/e*	1272	984	696	654	510	408	366	348	331	306	289	264	246	220	186
			rel intensity	(0.3)	4	3.3	5.0	9	100	89	32	20	22.5	24	15.2	42	15	21
	18	Laminaritetraose	*m/e*	408	366	348	331	318	306	270	258	246	220	192	186	167	150	125
			rel intensity	9.5	100	13	33	38	22	10	12	40	30	13	40	21	18	10
	19	Stachyose	*m/e*	1272	984	696	654	534	408	366	338	331	306	292	264	246	204	126
			rel intensity	100	63	41	31	10.4	12	30	13.5	34	46	41	11.5	58	14	22
	20	Nystose	*m/e*	1272	984	696	594	408	380	366	348	331	306	246	192	186	170	128
			rel intensity	(0.6)	29	100	13	65	5.2	13.3	18.5	41	9.5	12.2	6.1	35	17.6	5.7
Penta-saccharides	21	Mannopentaose	*m/e*	1272	984	694	654	408	366	331	303	279	255	220	186	167	150	108
			rel intensity	(0.72)	15.4	7.2	10.8	21	20	9	28	42	8	40	11	100	69	17
	22	Laminaripentaose	*m/e*	984	696	654	408	366	348	331	306	289	264	246	220	204	192	186
			rel intensity	6.2	9	7.5	28	100	53	10.5	34	20	7	47	9.7	6.1	7	23

[a] Only the 15 most intense ions below and including the molecular AIC are reported. Intensities in parentheses were not among the 15 most intense ions.

and **16**. The *m/e* 907 ion was observed only in the spectrum of **11** (1% relative abundance). This reflects the tendency for cleavage of the largest possible neutral fragment in all of these spectra. The ion at *m/e* 619 was also of low intensity (1–2%) in the three spectra where it appeared (**11**, **12**, and **14**). On the other hand the monoglycosyl ion, *m/e* 331, was prominent in virtually all of the spectra. This ion or its relatives, like *m/e* 259 in the spectrum of **10**, can be reliably used to establish the nature of the nonreducing end of an oligosaccharide.

Thermolysis of trisaccharide peracetates gives partial and peracetate derivatives of di- and monosaccharides, in addition to low-intensity ions at *m/e* 942, 924, 882, and 822 which correspond to loss of acetic acid and ketene residues from the molecular AIC. The mass spectrum of cellotriitol peracetate (**16**) contained thermolysis fragment AIC's from both the reducing and nonreducing sides of the molecule, although the ions from the reducing end of the trisaccharide were the most intense. This is in accord with similar observations for the disaccharide acetates **9** and **10**. The *m/e* 452 ion in the spectrum of **16** decomposes with loss of ketene to the *m/e* 410 ion (2.3%) as shown by an appropriate metastable ion.

16

Of the tetrasaccharides, only stachyose peracetate (**19**) showed an intense molecular AIC. For the peracetates of mannotetraose and nystose (**17** and **20**), this peak was of low intensity. In the spectrum of laminaritetraose peracetate (**18**), the molecular AIC was not observed. The general character of the fragmentation of the tetrasaccharide peracetates is very reminiscent of their trisaccharide relatives; however, in this case pyrolytic fragmentation is even more important, and *m/e* 331 and its decomposition products are the only significant nitrogen-free ions in the spectra. Ions which correspond to thermolytic cleavage of glycosyl linkages are generally more prominent for the tetrasaccharides than for their smaller relatives. For example, in the peracetylstachyose spectrum the ions corresponding to peracetyl tri-, di-, and monosaccharide AIC's had intensities of 63, 41, and 12%, respectively. Ions which corresponded to loss of ketene from *m/e* 696 and 408 (*i.e.*, *m/e* 654 and 366) were also prominent in the spectrum. Ammonium ion complexes of both of the disaccharide fragments of the tetrasaccharide peracetate can be seen in the spectrum, although the one which corresponds to the normal peracetyl disaccharide AIC, *m/e* 696 (41%), is considerably more intense than the ion arising from ammonium ion attachment to the other portion of the molecule, *m/e* 594 (6%). The reason for this probably stems from the relative thermal lability of the dehydrodisaccharide peracetate. It is conceivable that these unstable thermolysis products were further degraded and give rise to the ions at *m/e* 246, 220, and 192. It is also possible that these ions could have arisen from ammonia attachment to glycosyl fragment ions as in the case of *m/e* 186.

The ammonia–isobutane CI mass spectra of the pentasaccharide peracetates do not show a molecular AIC. The largest observable fragments correspond to a tetrasaccharide acetate for **21** and a trisaccharide acetate for **22**. The fact that pentasaccharide peracetates apparently cannot be vaporized without thermolysis will be a limitation on this method for molecular weight determination and structure analysis.

Summary. The presence of an intense molecular AIC in the ammonia–isobutane CI spectra of the peracetates of tetra-, tri-, and disaccharides will permit unequivocal molecular weight determinations. The existence of AIC's of thermolysis fragments in these spectra also allows detection of the nature of the individual monosaccharides in the chain (hexose, pentose, etc.). The mass of the glycosyl ion and the glycosydic fragment AIC's provides clear evidence concerning the nature of the reducing and nonreducing sugars in the chain. Because of the sensitivity of the spectra to source conditions it does not seem possible to use these spectra for stereochemical investigations.

The results obtained thus far suggest that alditols should be more useful in future sequencing experiments than the parent reducing sugars. If the permethyl ethers were used instead of the peracetates, the compounds should have a higher vapor pressure and be less sensitive to thermolysis. With these derivatives it might then be possible to take advantage of the relative selectivity of CI mass spectra for obtaining detailed sequence information on unknown oligosaccharides.

Experimental Section

The oligosaccharide acetates used in this study were analytical samples prepared by standard methods.[6] The spectra were obtained by use of a solid probe inlet with an AEI MS-902 mass spectrometer equipped with an SRIC chemical ionization source and a Mensor quartz monometer. All of the spectra were obtained with a 2:1 mixture of ammonia and isobutane at a total source pressure of ⅓ Torr (40 pascals). The source temperature was 250°. Small changes in source temperature generally had profound effects on the abundance of low-intensity ions in the spectra. Increasing the temperature decreased the prominence of adduct ions above the molecular AIC and increased the prominence of the thermolysis ions in the spectra.

Registry No.—**1**, 49587-30-6; **2**, 49587-31-7; **3**, 49587-31-7; **4**, 20880-60-8; **5**, 49587-33-9; **6**, 23846-69-7; **7**, 49587-35-1; **8**, 126-14-7; **9**, 49587-36-2; **10**, 49587-37-3; **11**, 49587-38-4; **12**, 49587-39-5; **13**, 25101-98-8; **14**, 32590-21-9; **15**, 6424-12-0; **16**, 49587-40-8; **17**, 49587-41-9; **18**, 49587-42-0; **19**, 6799-30-0; **20**, 25101-99-9; **21**, 49587-43-1; **22**, 49587-44-2.

References and Notes

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Molecular Design by Cycloaddition Reactions. VIII.[1] Syntheses of σ-Tris- and σ-Tetrakis(homobenzenoid) Skeletons by Carbene Additions to Medium-Membered-Ring Unsaturated Compounds

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Addition of dichlorocarbene in aqueous medium to medium-membered-ring unsaturated compounds was shown to proceed in a highly stereospecific manner. Thus, cyclooctatetraene and tropone ethylene ketal gave *all-trans*-σ-tetrakis(homocyclooctatetraene) and σ-tris(homotropone) ethylene ketal derivatives together with partly dichloromethylenated products, respectively. *N*-Ethoxycarbonyl-1(1*H*)-2,3- and -4,5-homoazepine afforded the corresponding *all-trans*-σ-tris(homoazepine) derivatives. The structural elucidation of these products was accomplished by spectral evidences and chemical properties. Possible mechanisms for these reactions are also discussed.

Recently, considerable interest has been shown in the chemistry of σ-trishomobenzene derivatives. Initially Prinzbach reported the syntheses of *cis*-oxa-σ-tris(homobenzene) and *cis*- and *trans*-aza-σ-tris(homobenzene) derivatives.[2] Elegant syntheses of *cis*-[3,4] and *trans*-trioxa-σ-tris(homobenzene)[5] and *trans*-σ-tris(homobenzene) derivatives,[6] and, furthermore, the thermally allowed $3\sigma \rightarrow 3\pi$ isomerization of the cis isomer have been reported.[3,4,7] We wish to report that carbene addition reactions to medium-membered ring unsaturated compounds offer a one-step syntheses of σ-tris- and σ-tetrakis(homobenzenoid) skeletons.