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CHARACTERIZATION OF *O*-TRIMETHYLSILYL DERIVATIVES OF D-GLUCOSE, D-GALACTOSE, AND D-MANNOSE BY GAS–LIQUID CHROMATOGRAPHY–CHEMICAL-IONIZATION MASS SPECTROMETRY WITH AMMONIA AS REAGENT GAS*

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ABSTRACT

The per(trimethylsilyl) ethers of D-glucose, D-galactose, and D-mannose were analyzed by g.l.c.–c.i.m.s. with ammonia as the reagent gas. C.i.m.s. gave simple fragmentation and fragment ions of high intensity in the high-mass range where the QM^+ ion is also detected. The β-D anomers gave ions at *m/e* 558 showing intensities 3–12 times those of the α-D anomers. The epimers could be distinguished by differences in the intensities of the ions and by the observation that D-glucose gave a base peak at *m/e* 198, D-galactose at *m/e* 468, and D-mannose at *m/e* 204. The pyranose and furanose structures could be distinguished by comparing the ion intensities at *m/e* 198, *m/e* 271, *m/e* 361, *m/e* 396, and *m/e* 451. A similar analysis was also performed with 2-methylpropane as the reagent gas.

INTRODUCTION

The electron-impact mass spectrometry (e.i.m.s.) of monosaccharide derivatives, such as *O*-methyl[1,2], *O*-acetyl[3], *O*-isopropylidene[4,5], *O*-trimethylsilyl[6–8], and *O*-trifluoroacetyl[9] derivatives, has been extensively investigated. Of special interest is the technique of deuterium labeling and exact mass determination to study the minute fragmentations of the *O*-trimethylsilyl derivatives, and the use of the difference in the ion intensities of the *m/e* 204 and *m/e* 217 ions to differentiate between furanose and pyranose structures[6]. Ando *et al.*[10] studied the mass spectrum of the *O*-trifluoroacetyl derivatives of methyl hexopyranosides to differentiate between anomers and epimers. The disadvantages of the e.i.m.s. technique is that the ion intensities are rather low in the high-mass range where molecular ions are detected, and that it cannot clearly differentiate between such stereoisomers as anomers and epimers. This is most conspicuous with the *O*-trimethylsilyl derivatives, which are the most

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commonly used. Hogg and Nagabhushan[11] analyzed, by chemical-ionization mass spectrometry (c.i.m.s.) in the presence of ammonia as the reagent gas, D-glucose, methyl α-D-glucopyranoside and β-D-glucopyranoside, and *O*-acetyl derivatives of α,α-trehalose; they detected the $(M + NH_4)^+$ ion and showed that molecular weights could easily be detected. They were also able to follow the fragmentation of the protonated molecular ion of ^{13}C-labeled acetates when methane was used as the reagent gas. The new technique of chemical-ionization mass spectrometry[12,13] has not been reported, as yet, for the *O*-trimethylsilyl derivatives of monosaccharides.

In the present paper, we describe the analysis by gas–liquid chromatography–chemical-ionization mass spectrometry (g.l.c.–c.i.m.s.) of the *O*-trimethylsilyl derivatives of D-glucose, D-galactose, and D-mannose with ammonia as the reagent gas. It was found that the molecular weights are easily determined, that the ion intensity is high in the high-mass range, that the fragmentation is simple, and that the fragment ions are produced in a quantity high enough to differentiate between stereoisomers (epimers and anomers).

RESULTS AND DISCUSSION

The gas–liquid chromatogram of the *O*-trimethylsilyl derivatives of D-galactose

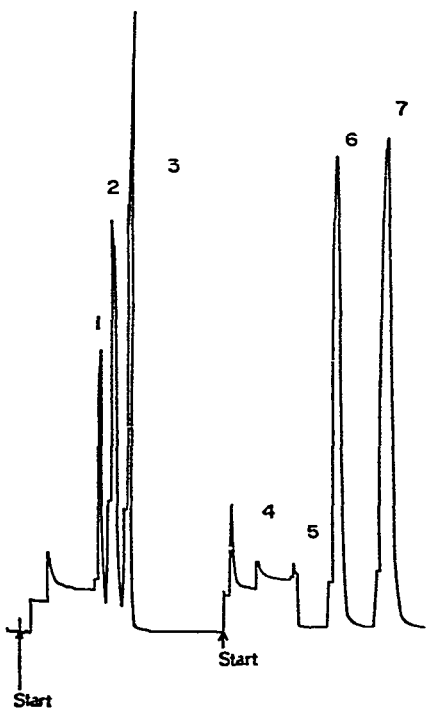


Fig. 1. Gas–liquid chromatograms of the per-*O*-trimethylsilyl derivatives of D-galactose and D-glucose: (1) α-D-galactofuranose (1), (2) α-D-galactopyranose (2), (3) β-D-galactopyranose (3), (4) not identified, (5) α-D-glucofuranose (4), (6) α-D-glucopyranose (5), and (7) β-D-glucopyranose (6).

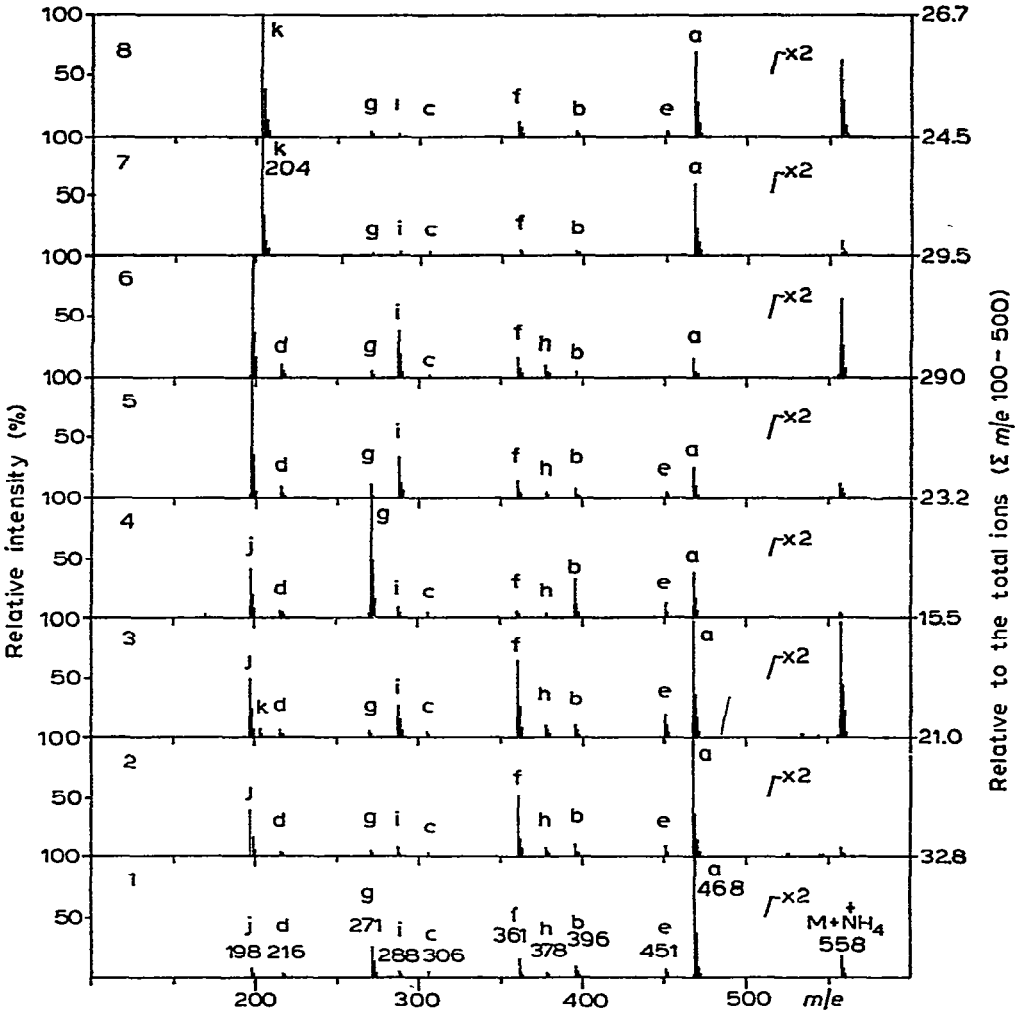
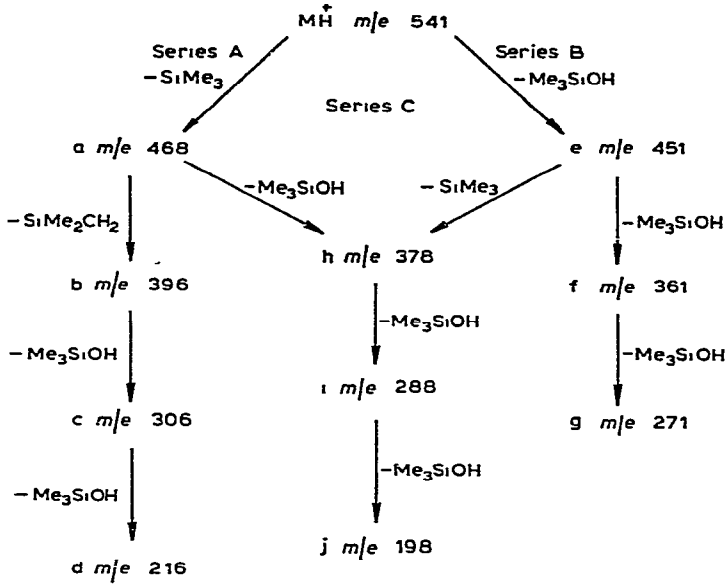


Fig. 2. Chemical-ionization mass spectra of the per-*O*-trimethylsilyl derivatives of D-galactose, D-glucose, and D-mannose.

shows three peaks (P-1, P-2, and P-3), and that of D-glucose four peaks (P-4, P-5, P-6, and P-7) (Fig. 1). The corresponding mass spectra are presented in Fig. 2, which also includes the mass spectra of *O*-trimethylsilyl derivatives of D-mannose. Table I shows the ratios of the fragment ions relative to the total ion, and the relative intensities.

Chemical-ionization mass spectra. — We confined our study to the significant fragment ions of c.i.m.s., as a satisfactory study by e.i.m.s. has been published[6]. The quasi-molecular ions (QM^+) is recorded at *m/e* 558 [$M + 18 = (M + NH_4)^+$], ammonia being the reagent gas. The fragmentations are classified into series A, B, and C (Scheme 1). Series A starts from the *m/e* 468 ion ($MH^+ - SiMe_3$). The *m/e*



Scheme 1. Chemical-ionization mass fragmentation of series A, B, and C.

396 ion is produced by elimination of $SiMe_2C^+H_2$, the *m/e* 306 ion is produced by elimination of one Me_3SiOH fragments, and the last ion (*m/e* 216) is produced by elimination of one Me_3SiOH fragment. Series B starts from the *m/e* 451 ion (MH^+ — Me_3SiOH). The Me_3SiOH fragments are eliminated successively to produce the *m/e* 361 ion (two Me_3SiOH fragments have been eliminated from the MH^+ ion), and the *m/e* 271 ion (three Me_3SiOH fragments have been lost). Series C starts from the *m/e* 378 ion, which has been produced from the *m/e* 468 ion of the series A by elimination of one Me_3SiOH fragment or from the *m/e* 451 ion of the series B by elimination of one Si^+Me_3 fragment. The next ion in the series C is the *m/e* 288 ion, produced by elimination of one Me_3SiOH fragment, and then the *m/e* 198 ion is produced by elimination of two Me_3SiOH fragments. The last fragment ions of the three series are the *m/e* 216, *m/e* 271, and *m/e* 198 ions, respectively. D-Mannose gave, at *m/e* 204, a base peak that was less intense than those for D-glucose and D-galactose. This peak is very characteristic for the pyranose structure in e.i.m.s.

Differentiation of anomers. — Anomers are difficult to differentiate by e.i.m.s. of the *O*-trimethylsilyl derivatives. Ando *et al.*[10] reported that, in the e.i.m.s. of the trifluoroacetyl derivatives, the molecular ion of the β-D anomer has always about twice the intensity of that of the corresponding α-D anomer.

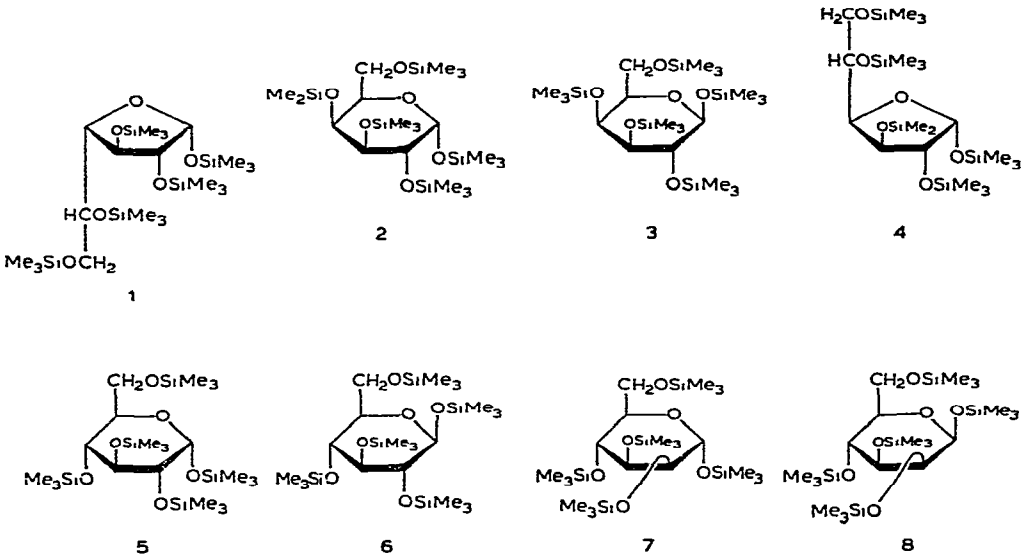
In c.i.m.s., however, 1,2,3,4,6-penta-*O*-trimethylsilyl-α-(**2**) and β-D-galactopyranose (**3**) gave a base peak at *m/e* 468 and a quite similar profile of the peak range. The only difference resided in the relative intensity of the $(M + NH_4)^+$ ion of **3** showing a much more intense peak than that of **2**. The intensity of the $(M + NH_4)^+$ ion, relative to the total ion-intensity, was 1% for **2** and 8.8% for **3**. Relative

TABLE I

TOTAL AND RELATIVE INTENSITY OF IMPORTANT IONS OF PER-*O*-TRIMETHYLSILYL DERIVATIVES (1–7) OF D-GALACTOSE AND D-GLUCOSE

Ions[a]	m/e	*Compounds*													
		1		2		3		4		5		6		7	
		T[b]	*R*[c]	*T*	*R*	*T*	*R*	*T*	*R*	*T*	*R*	*T*	*R*	*T*	*R*
$(M + NH_4)^+$	558	3.4	10.2	1.0	4.5	8.8	56.3	1.8	5.2	0.8	3.2	2.3	8.2	7.8	26.6
a	468	32.8	100.0	21.0	100.0	15.5	100.0	34.5	100.0	8.2	35.5	7.7	28.0	5.5	18.6
e	451	4.1	12.4	3.4	16.3	3.2	20.3	0.4	1.1	3.5	15.2	0.8	2.9	—	—
b	396	4.2	12.9	1.9	8.8	1.4	8.9	0.8	2.1	6.5	28.0	2.2	8.0	7.5	5.0
h	378	0.5	1.5	1.4	6.8	1.2	9.4	0.7	1.8	0.5	2.1	1.7	6.3	1.4	4.8
f	361	5.7	17.4	10.2	48.8	10.3	66.2	1.7	4.9	2.2	9.4	5.3	19.3	4.4	15.0
c	306	1.4	4.2	1.2	5.7	1.0	6.3	2.1	5.9	1.0	4.1	0.9	3.4	0.6	2.1
i	288	0.4	1.0	4.4	20.8	4.5	28.8	1.2	3.5	4.8	20.5	10.8	39.5	10.9	36.9
g	271	7.6	23.2	1.7	8.0	0.5	3.1	—	—	23.1	100.0	3.4	12.7	1.3	4.3
d	216	1.2	3.5	1.0	4.7	1.1	7.3	12.7	36.7	2.4	10.1	2.7	9.7	2.9	9.7
j	198	2.3	6.9	8.7	41.2	9.8	63.0	2.8	8.1	10.1	43.3	27.4	100.0	29.5	100.0

[a]Quasi-molecular ion and fragment ions. [b]T, Percentage relative to the total ion intensities (Σ *m/e* 100 – 500). [c]R, Relative intensity.



to the intensity of the base peak (100), the intensity of the $(M + NH_4)^+$ ion was 4.5% for **2** and 56.3% for **3**.

1,2,3,4,6-Penta-*O*-trimethylsilyl-α-(**5**) and -β-D-glucopyranose (**6**) gave a base peak at *m/e* 198. Like the D-galactose derivatives, the D-glucose derivatives gave similar profiles of fragment ions in the mass-range lower than *m/e* 468. The β-D anomer **6** gave a more intense $(M + NH_4)^+$ ion than did **5**. The intensity of the $(M + NH_4)^+$ ion, relative to the total ion intensity, was 2.3% for **5** and 7.8% for **6**. Relative to the intensity of the base peak (100), the intensity of the $(M + NH_4)^+$ ion was 8.2% for **5** and 26.6% for **6**. The *g* ions (*m/e* 271) showed some different intensities, but none so conspicuous as the $(M + NH_4)^+$ ions, the intensities of the ions of the α-D-anomers being 3–4 times greater than those of the corresponding β-D anomers, in both D-galactose and D-glucose derivatives.

1,2,3,4,5-Penta-*O*-trimethylsilyl-α-(**7**) and -β-D-mannopyranose (**8**) gave results similar to those of the D-galactose and D-glucose derivatives. Thus, the difference in abundance of the $(M + NH_4)^+$ ions for D-galactose, D-glucose, and D-mannose may be caused by the steric effects of the configuration of the trimethylsilyloxy group at C-1.

The intensity of the $(M + NH_4)^+$ ion relative to the total ion-intensity was examined against various operational conditions of c.i.m.s.; it was found that this intensity was the highest when the ion-source temperature was 180° and the pressure of the NH_3 gas 0.9 torr, hence showing the greatest difference between the anomers; under any operational conditions the intensity of the β-D anomer was the greater.

Differentiation of epimers. — The e.i.m.s. data of methyl ethers[4], *O*-isopropylidene derivatives[4], and trifluoroacetates[10] have been studied for differences between epimers. No significant difference in intensity from the high-mass to the

low-mass range was observed, either for these derivatives or for the *O*-trimethylsilyl derivatives[9].

In c.i.m.s., however, 1,2,3,4,6-penta-*O*-trimethylsilyl-D-galactose gave the base peak *a* at *m/e* 468 and the D-mannose derivative gave a base peak *k* at *m/e* 204, in contrast with the base peak *j* at *m/e* 198 of the D-glucose derivative. D-Galactose, D-mannose, and D-glucose derivatives gave the same fragment ions, except the ion *k*. But the relative intensities of these ions were all different, except for the ions *b* and *h* (see Table I and Fig. 1).

The configuration at C-1 plays no role in the differentiation between epimers, since α-D-glucopyranose and β-D-glucopyranose derivatives, and α-D-galactopyranose and β-D-galactopyranose derivatives gave, respectively, similar mass spectra, except for the $(M + NH_4)^+$ ion.

Furanose derivatives **1** *and* **4**. — In the chromatogram (see Fig. 1), the peak P-1 for the D-galactose derivative and the peak P-5 for the D-glucose derivative correspond to a furanose structure. They were identified by DeJongh *et al.*[6] as corresponding to the derivatives of α- or β-D-galactofuranose, and α- or β-D-glucofuranose, respectively. In c.i.m.s., the pentakis(trimethylsilyl) ethers of D-galactose gave a base peak at *m/e* 468 (*a*), but the ratio to the total ion intensity was 15.5% for **2**, 21.0% for **3**, and 32.8% for **1**. The 1,2,3,4,6-pentakis(trimethylsilyl) ether of α-(**5**) and β-D-glucopyranose (**6**), gave a base peak at 198, whereas the furanose **4** gave a base peak at *m/e* 271 (*g*). Another noticeable difference is the greater intensity of the ion *g* for **1** as compared to that of **2** and **3**, whereas the intensity of the ions *f*, *i*, and *j* was lower for **2** and **3**, as compared to that of **1**. The intensity of the $(M + NH_4)^+$ ion relative to the total ion intensity was 3.4% for **1** and 0.8% for **4**, and the intensity relative to the base peak was 10.2% for **1** and 3.2% for **4**. Thus, like the α-D anomers of the pyranoses (**4** and **5**), the α-D anomers of the furanoses give a low ion-intensity. This suggests that **1** and **5** are the α-D-galactofuranose and α-D-glucofuranose derivatives, respectively, although no comparison could be made with the β-D anomer.

The epimers **1** and **4** could be differentiated by comparing the ions *m/e* 198 (*j*), *m/e* 271 (*g*), and *m/e* 468 (*a*). The g.l.c. of the D-glucose derivatives gave an additional P-4 (Fig. 1); its $(M + NH_4)^+$ ion had a low intensity, and the fragment ions were the same as those given by peaks P-1–P-6. As for the D-galactose derivatives, *m/e* 468 (*a*) was detected as the base peak, but ions *g* and *d* gave different intensities, and the retention time of P-4 did not coincide with the retention time of any of the peaks given by D-galactose derivatives. Thus, the P-4 peak could not be identified.

Analysis of per(trimethylsilyl) ethers of D-*glucose and* D-*galactose with 2-methylpropane as reagent gas.* — In this analysis, 2-methylpropane replaced ammonia as the reagent gas. The furanose and pyranose forms could be differentiated both for the D-glucose and D-galactose derivatives, the furanose form giving a high ion-intensity at *m/e* 271, and the pyranose form a high ion-intensity at *m/e* 361.

The anomers could also be easily differentiated by comparing the QM^+ ion, $(M - H^+)$ at *m/e* 539 and $MH^+ - CH_4$ at *m/e* 525. The fragment ions at *m/e* 539 and

m/e 525 gave intensities of only 2–4% of that of the base peak at *m/e* 361. The β-D anomer, however, gave a high ion-intensity at *m/e* 539 and no ion or an ion of very low intensity, if any, at *m/e* 525. The α- and β-D anomers could be differentiated by examination of the QM^+ ion and ($MH^+ - CH_4$) ions in the same manner as with ammonia as the reagent gas.

The epimers could be differentiated by the ions at *m/e* 232 and *m/e* 244, the D-glucose derivative giving, at *m/e* 244, an ion having an intensity higher than that of the ion at *m/e* 232, whereas the D-galactose derivative gave an ion having a lower intensity at *m/e* 244. The ammonia gas gave better results for differentiating anomers and epimers than did 2-methylpropane. 2-Methylpropane could also serve for differentiating the D-galactose and D-glucose derivatives having furanose structures (**1** and **4**); **1** gave a highly intense ion at *m/e* 361, besides the base peak at *m/e* 271. Both **1** and **4** could be identified from the ratio of the ($M - H^+$) ion and ($MH^+ - CH_4$) ion.

EXPERIMENTAL

Materials. — D-Glucose (0.1 mg), D-galactose (0.1 mg), and D-mannose (0.1 mg) were obtained from Wake Chemicals Co. Ltd. (Kyoto, Japan) and were equilibrated in water for about 12 h.

Per-O-trimethylsilylation. — D-Galactose, D-glucose, and D-mannose were completely converted into the per-*O*-trimethylsilyl ethers by the method of Sweeley *et al.*[14].

Gas-liquid chromatography–chemical-ionization mass spectrometry. — The analyses were performed with a Shimadzu LKB 9000 gas chromatograph–chemical-ionization-source mass spectrometer–computer combined system (Shimadzu Seisakusho Ltd., Nakagyo-ku, Kyoto, Japan). The data-processing system (GCMS-PAC 300DG) consisted of an OKITAC-4300C minicomputer with 12K core, a typewriter, an incremental plotter, a magnetic disc, and an interface to the g.l.c.–m.s. instrument. The gas-liquid chromatograph was equipped with a glass column 1.5m × 3mm (i.d.) containing 2% of OV-17 on Chromosorb W (80–100 mesh), kept at 160°. The mass-spectrometric data were obtain with an ion-source temperature of 180°, a 500-eV electron energy, a 500-μA emission current, and a 3.5-kV acceleration voltage. The reagent gases were ammonia and 2-methylpropane, at a pressure of the ionization source of 0.9 and 0.6 torr, respectively.

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