CHROM. 11,021

Note

Gas–liquid chromatographic separation of monomethylguanines as their trimethylsilyl derivatives*

GARY M. MUSCHIK** and WAYNE B. MANNING

Chemical Carcinogenesis Program, NCI Frederick Cancer Research Center, Frederick, Md. 21701 (U.S.A.)

A number of workers[1–10] have reported gas–liquid chromatography (GLC) data for purine and pyrimidine bases, nucleosides and nucleotides but data for only a few monomethylated derivatives have been reported. This note reports a method employing GLC for the separation of silyl derivatives of mono-methylated guanines, and describes this separation.

EXPERIMENTAL

A Varian Model 1440 gas–liquid chromatograph equipped with a flame-ionization detector and a linear-temperature programmer was used for this study. Helium carrier-gas flow-rate was 48 ml/min, air flow-rate was maintained at 200 ml/min and the hydrogen flow-rate was 20 ml/min as measured by a soap-bubble flow-meter. Sample injection volumes were 1–2 μl. The chromatographic columns were either 5.7% (w/w) SE-30 on Chromosorb W HP (100–120 mesh) or 5% (w/w) OV-3 on Chromosorb W HP (100–120 mesh), packed in glass columns 6 ft. × 2 mm I.D. The column temperatures were programmed from 150 to 275° at 6°/min.

A Finnigan series 1015C CI-EI GLC–mass spectrometry (MS) apparatus equipped with a chemical ionization (CI) source and interfaced with a Finnigan 6000 MS data system was used to collect the MS data. All spectra were collected at an ionization potential of 130 eV with methane as the CI gas.

The monomethylguanines were obtained commercially except for O^6-methyl-guanine that was prepared by the method of Balsiger and Montgomery[11] and 8-methylguanine which was prepared by the method of Daves *et al.*[12]. N,O-Bis-(tri-methylsilyl)acetamide (BSA) was obtained from Sigma (St. Louis, Mo., U.S.A.).

The trimethylsilyl (TMS) derivatives of the methylguanines were prepared as follows. A stock solution containing 2.7 mg of phenanthrene (internal standard), 4 ml of acetonitrile and 0.1 ml of BSA was prepared as the silylating reagent. Known amounts (0.1–0.3 mg) of each of all monomethylguanines were placed in a micro

---

reaction vessel (Supelco, Bellefonte, Pa., U.S.A.), along with known volumes (0.1–0.15 ml) of the above silylating reagent. The micro reaction vessels were placed into a 130° oil bath for 90 min and then analyzed by GLC.

RESULTS AND DISCUSSION

The conversion of the monomethylguanine isomers to their respective TMS derivatives resulted in a volatile and thermally-stable derivative for GLC analysis and gave a fast and sensitive method for the analysis of these compounds.

The advantages and limitations of this GLC method have been examined using SE-30 and OV-3 liquid phases. A comparison of relative retention values on the SE-30 liquid phase with those obtained on the OV-3 liquid phase was made and appears in Table I. The SE-30 liquid phase had the distinct advantage of separating all mono-methylguanine-TMS derivatives (Fig. 1). Lakings et al.[7] reported that the separations of a broad spectrum of commercially-available methylated bases were best achieved on OV-3. However, our findings indicate that SE-30 gave better resolution for the separation of monomethylguanines. Neither SE-30 nor OV-3 could separate guanine from 1-methylguanine-TMS derivatives. A disadvantage of OV-3 was its inability to resolve the O^6-methylguanine, 1-methylguanine and guanine-TMS derivatives. In contrast, SE-30 resolved O^6-methylguanine from either 1-methylguanine or guanine TMS derivatives. The mixture of 7-methylguanine and 8-methylguanine TMS derivatives was also unresolved on OV-3. However, a mixture consisting of the TMS derivatives of N^2-methylguanine, O^6-methylguanine, 7-methylguanine, and 9-methylguanine was better resolved on OV-3 than on SE-30. We found that satisfactory resolution of all volatile monomethylguanine derivatives could be achieved on SE-30.

The number of TMS groups per monomethylguanine molecule (Table I) was determined from the GLC–CI–MS quasi-molecular ion values of the product formed under silylating conditions which resulted in complete derivatization. Guanine and 8-methylguanine gave tri-TMS derivatives. Di-TMS derivatives were observed for 1-

TABLE I

RETENTION TIMES AND NUMBER OF SILYL GROUPS FOR THE RESPECTIVE MONO-METHYLGUANINE-TMS DERIVATIVES

α = Relative retention time (phenanthrene = 1.00).

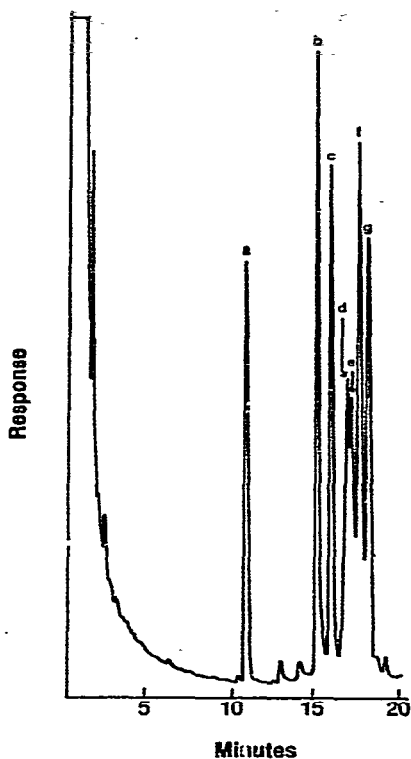| Compound	TMS retention times				Number of silyl groups
	OV-3		SE-30		
	Time (min)	α	Time (min)	α	
Guanine	22.1	1.75	17.9	1.53	3
1-Methylguanine	22.1	1.75	17.4	1.49	2
N^2-Methylguanine	20.0	1.59	16.4	1.40	2
3-Methylguanine	—		—		—
O^6-Methylguanine	22.1	1.75	17.3	1.48	2
7-Methylguanine	23.3	1.85	18.1	1.55	2
8-Methylguanine	23.6	1.87	18.7	1.60	3
9-Methylguanine	19.0	1.51	15.2	1.30	2
Phenanthrene	12.6	1.00	11.7	1.00	—

Fig. 1. Separation of monomethylguanine-TMS derivatives on SE-30. a = phenanthrene (internal standard); b = 9-methylguanine; c = N²-methylguanine; d = O⁶-methylguanine; e = 1-methylguanine; f = 7-methylguanine; g = 8-methylguanine.

methylguanine, N^2-methylguanine, O^6-methylguanine, 7-methylguanine, and 9-methylguanine, as might be anticipated from their respective structures. The di-TMS structure for 7-methylguanine and the tri-TMS structure for guanine were observed previously and reported by electron impact MS by Hattox and McCloskey[13]. The GLC profiles and GLC–CI–MS molecular ion data on samples prepared under incomplete reaction conditions showed the presence of both di-TMS and tri-TMS derivatives for guanine and 8-methylguanine; both mono-TMS and di-TMS derivatives for 9-methylguanine. These respective mono-, di- and tri-TMS derivatives were observed to have different GLC relative retention (α) values. It is therefore necessary in qualitative and quantitative analysis of these compounds, to generate the completely silylated products. For example, the di-TMS derivatives of guanine and 9-methylguanine had approximately the same relative retention values on the SE-30 column. The GLC lower detection limit, expressed in nanograms of purine, providing discernible and useful peaks for quantitative measurements ranged from about 2 ng for guanine to 20 ng for 1-methylguanine.

This method has been useful in separating standard mixtures of monomethylguanines, the monomethylguanines obtained from hydrolysis of methylated guanosine, and the monomethylguanines from hydrolysis of methylated polyguanylic acid (Fig. 2).
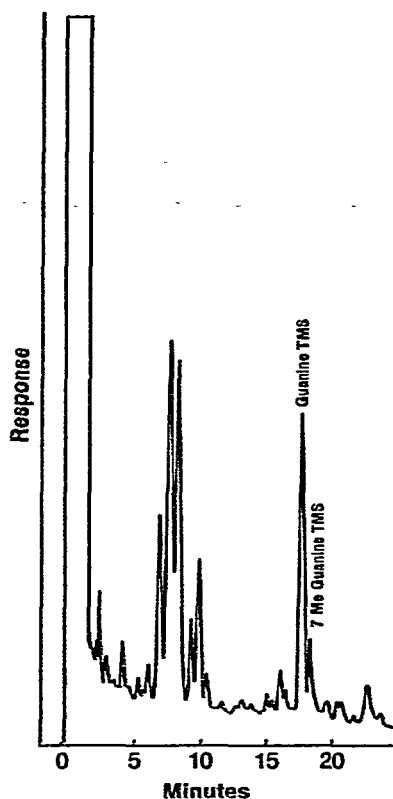
Fig. 2. Analysis of TMS derivatives from the hydrolysis of methylated polyguanylic acid. Methylation conditions: polyguanylic acid, dimethylsulfate, 37°, 18 h, phosphate buffer pH = 7.0. Hydrolysis conditions: hydrochloride, 100°, 1 h. Silylation conditions: see Experimental.

REFERENCES

1 I. A. Muni, C. H. Altschuler and J. C. Neicheril, *Anal. Biochem.*, 50 (1972) 354.
2 W. C. Butts, *Anal. Biochem.*, 46 (1972) 187.
3 W. C. Butts, *J. Chromatogr. Sci.*, 8 (1970) 474.
4 R. L. Hancock, *J. Chromatogr. Sci.*, 7 (1969) 366.
5 R. L. Hancock, *J. Gas Chromatogr.*, 4 (1966) 363.
6 D. B. Lakings and C. W. Gehrke, *Clin. Chem.*, 18 (1972) 810.
7 D. B. Lakings, C. W. Gehrke and T. P. Waalkes, *J. Chromatogr.*, 116 (1976) 69.
8 S. Y. Chang, D. B. Lakings, R. W. Zumwalt and C. W. Gehrke, *J. Lab. Clin. Med.*, 83 (1974) 816.
9 C. W. Gehrke, D. L. Stalling and C. D. Ruyle, *Biochem. Biophys. Res. Commun.*, 28 (1967) 869.
10 C. W. Gehrke and D. B. Lakings, *J. Chromatogr.*, 61 (1971) 45.
11 R. W. Balsiger and J. A. Montgomery, *J. Org. Chem.*, 25 (1960) 1573.
12 G.12 G. D. Daves, Jr., C. W. Noell, R. K. Robins, H. C. Koppel and A. G. Beaman, *J. Amer. Soc.*, 82 (1960) 2633.
13 S. E. Hattox and J. A. McCloskey, *Anal. Chem.*, 46 (1974) 1378.