Defence Science Journal, Vol. 62, No. 5, September 2012, pp. 319-323, DOI: 10.14429/dsj.62.1167

Gas Chromatographic Retention Indices of 2-chlorobenzylidenemalononitrile and its Analogues

P.K. Gutch* and R.K. Srivastava

Defence Research and Development Establishment, Gwalior–474 002, India
**E-mail: pkgutch@rediffmail.com*

ABSTRACT

Riot control agent, 2-chlorobenzylidenemalononitrile (CS) and its analogues have both skin irritating and lacrymating properties. Herein, we report retention indices (RI) of CS and its thirteen analogues relative to the homologues n-alkanes series. These values are determined on nonpolar BP-1 and polar BP-10 capillary column under programmed temperature and isothermal chromatographic condition. The analogues differ in substitution at *ortho* or *para* position of phenyl ring and retention indices are found to vary according to the nature of the substituent.

Keywords: Chemical warfare agents, riot control agents, retention indices, gas chromatography

1. INTRODUCTION

Chemical warfare agents can be classified into two general categories, those that exert a lethal effect and those that act in an incapacitating manner. Lethal chemical warfare agents include nerve agents such as Sarin, Soman, and Tabun, while incapacitating agents include irritants (tear gases or riot control agents). Acute exposure to irritants causes a number of incapacitating effects including burning or irritation of the skin and eyes, coughing, nausea and vomiting. The incapacitating nature of these chemicals has led to the development of dispersal devices for their use in riot control situations, during military training exercises and to a lesser extent as chemical weapons on the battlefield[1]. The most commonly employed irritants are o-chlorobenzylidenemalononitrile[2], often referred to as tear gas, and 2–chloroacetophenone[3]. Dibenz (b,f)–l,4-oxazepin[4] has been used less frequently and 1-methoxycycloheptatriene was evaluated as a possible military training agent.

Convention on the Prohibition[5] of the development, production, stockpiling and use of chemical weapons and their destruction states that, 'Each state party undertakes not to use riot control agents as a method of warfare'. United Nations peacekeeping forces could encounter the use of irritants during active duty in regions of the world where there is a threat of chemical warfare agent use. Intelligence gathering, through the collection of contaminated samples, and subsequent analysis of the samples would enable identification of the suspect chemical and confirm use of a controlled chemical for warfare purposes. The results of such analyses would likely contribute to the development of appropriate strategic and political positions. Gas chromatography (GC) methods, including methods based on GC retention indices[6], have been used for the detection of irritants in suspect samples[7-8]. These methods and others involving the use of chromatographic techniques for the detection of irritants and other chemical warfare agents have been reviewed.

Benzylidenemalononitriles (BMNs) are the main products of the condensation of substituted benzaldehydes with malonitrile[9-10]. Derivatives of benzylidenemalononitriles have important applications in various areas of chemistry. BMN derivatives which incorporate bis- (2–chloroethyl) amino groups, (bis–chloromethyl) pyrrolidine substituents or phenylazopyrimidine residue have been used for chemotherapeutic treatment of cancer[11]. Other substituted BMN compounds are used as pesticides, fungicides and insecticides[12]. The interest which have been shown in this group of compounds was largely due to use of 2-chlorobenzylidenemalononitrile (CS) as a riot control agent. CS causes irritation of eyes, nose, and respiratory tract with the consequent production of profuse tears and mucus. It is therefore, also termed as an irritant or tear gas compound[13].

The correlation of retention indices between an unknown and reference compound on two or more columns of different polarities is generally sufficient for identification purposes. For the evolution of retention indices, a homologues series of n-alkanes is commonly used as a reference compound. The measurement of Kovats retention indices[7] is useful only when compound with similar retention behavior are to be studied, as the column temperature in these measurements is held constant. Recently, temperature programmed retention indices using Van den Dool and Kratz equation have been found to be useful for compounds of defence interest[7, 14-15].

Methods for detection, identification and quantitative determination of CS and their analogues are required during their production and also for verification of their use of prohibited activities. The availability of identification data on CS and related compounds would facilitate the verification in case of alleged use of these chemicals. Herein, we report retention indices for CS and its 13 analogues determined under programmed temperature and isothermal GC conditions. Initially, retention indices[16] were determined on a polar BP-

Received 16 August 2011, revised 22 August 2012, , online published 14 September 2012

10 capillary column and then the effect of stationary phase polarity was studied by determining these values on a nonpolar BP-1 column.

2. EXPERIMENT

C_6-C_8 n-alkanes were purchased from BDH (Poole, U.K.) and C_9-C_{24} n-alkanes from Fluka (>99%, Bluchs, Switzerland). The solvents (methanol, acetonrtrile, and acetone) used for chromatographic analysis were of analytical grade. CS and its analogues were synthesized[16] in our laboratory by the condensation of substituted benzaldehydes with malononitrile in cyclohexane in the presence of piperdine as the base (Fig. 1). The BMNs were purified by recrystallisation and the purity of each compound was found to be 98-99 per cent by GLC analysis on column DB-I using temperature programming 40 °C to 200 °C, using N_2 as carriers gas. All the BMNs were characterised by elemental analysis, FTIR, NMR, and mass spectra.

Shimadzu (Model GC-9A) gas chromatograph equipped with a flame ionization detector (FID) and a CR3A integrator was used for the determination of retention indices of BMNs on two columns (30 m x 0.30 mm x 0.25 μ) of different polarities namely BP-1 (100% dimethyl polysiloxane) and BP-10 (14% cyanopropyl-phenyl dimethyl polysiloxane).

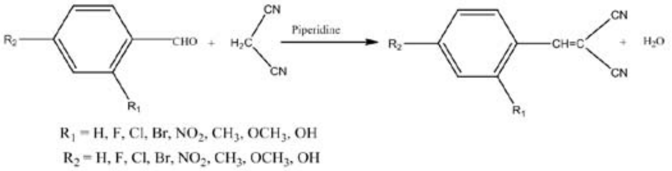

Figure 1. Synthesis of CS and analogues.

The column was programmed from an initial temperature of 40 °C (1 min) at the rate of 10 °C/min to final temperature of 280 °C (10 min). The injector and detector temperature was maintained at 230 °C. Nitrogen was used as a carrier gas at a pressure of 1.25 kg cm^{-2} at the inlet. Hydrogen and air were used as fuel gases at the pressures of 0.75 and 0.45 kg cm^{-2} respectively. Isothermal analysis was carried out at 200 °C.

3. DETERMINATION OF RETENTION INDICES

0.1 μl solution of BMNs (10-15 mg) in acetone together with n-alkane standard was injected on GC column and the retention times (accuracy ± 0.001 min.) were recorded. An authentic sample of each compound was also injected separately and their retention times were compared with that of the components in the mixture[16].

3.1 Under Programmed Temperature Conditions

Programmed temperature GC allowed the analysis of a number of compounds over a wide range of volatilities in a single run. Under programmed temperature conditions, a linear relationship exists between the retention time of n–alkanes and their carbon number. Hence, under these conditions, it is possible to calculate retention index value using retention time only. The retention indices under programmed temperature chromatographic condition (RI_p) were calculated using the van den Dool and Kratz formula. (Eqn (1))

$$RI_P = 100\left(\frac{t_c - t_z}{t_{z+1} - t_z}\right) + 100z \tag{1}$$

where t_c, t_z and t_{z+1} were retention times of the solute, alkane eluted immediately prior to the compound with z number of carbon atoms (lower alkane), and alkane eluted immediately after the compound with z+1 number of carbon atoms (higher alkane), respectively[7,17-18].

The RI_P valves determined on the polar BP-10 and non-polar BP-1 columns under different chromatographic condition are presented on Tables 1 and 3. Replacement of the *ortho* substituted chloro group from CS molecule by *-F*, *-H*, and *-CH_3* groups in analogues caused significant decrease in RI_p value in both BP-1 and BP-10 columns. This replacement led to a decreased interaction of the analyte molecule with the stationary phase and manifested in the retention time and

Table 1. Temperature programmed retention indices for *ortho* and *para* substituted benzylidenemalononitriles on BP-1 column

R_1	R_2	Molecular weight	Melting point °C)	R_t (min.)	Hydrocarbons (n+N) and their R_t	RI $_P$
F	H	172	119	19.62	C-14:19.385, C-15:20.88	1409.27
H	H	154	83	21.23	C-14:20.21, C-15:21.92	1472.78
Cl	H	188	94	21.985	C-15:20.88, C-16:22.225	1521.31
CH3	H	168	104	23.17	C-14:21.92, C-15:23.43	1670.79
Br	H	232	90	22.095	C-16:22.225, C-17:23.56	1624.59
OCH_3	H	184	84	24.175	C-17:23.97, C-18:25.25	1736.39
NO_2	H	199	143	24.79	C-17:23.97, C-18:25.25	1772.78
H	F	172	126	20.225	C-14:20.21, C-15:21.92	1433.14
H	Br	232	162	22.51	C-17:27.97, C-18:25.25	1650.56
H	CH_3	168	134	22.9	C-16:20.225, C-17:23.555	1764.8
H	Cl	188	188	24.655	C-16:20.225, C-17:23.555	1621.35
H	OCH_3	184	115	24.37	C-18:25.25, C-19:26.477	1810.19
H	NO_2	199	159	27.017	C-19:26.477, C-20:27.847	1936.42
H	OH	170	188	28.47	C-20:27.847, C-21:30.525	2020.92

retention index value of flouro, unsubstantiated BMN and nitro analogues in Tables 1 and 3. Similarly *para* substituted chloro, bromo, methyl, methoxy and nitro analogues resulted in a large increase in the RI_p values because of strong interactions between the analyte and the stationary phase. Significant changes in RI_p values took place with changes in the stationary phase polarity. There was a noticeable increase in RI_p value for each compound with increase column polarity (from BP-1 to BP-10 column) which was shown in Table 5.

3.2 Under Isothermal Chromatographic Conditions

Under isothermal conditions unlike programmed temperature conditions, there exists a non-linear relationship between retention time and number of carbon atoms of n-alkanes. Therefore, for the calculation of retention indices under these conditions, logarithm of the corrected of retention time was taken into account. Retention indices under isothermal experimental conditions (RI_I) were calculated using the Kovats formula (Eqn. (2)).

$$RI_I = 100\left(\frac{\log t'_c \;\; - \log t'_z}{\log t'_{z+1} \;\; - \log t'}\right) + 100z \qquad (2)$$

where t'_c, t'_z and t'_{z+1} were corrected retention times of the solute, alkane eluted immediately prior to the compound with z number of carbon atoms (lower alkane), and alkane eluted immediately after the compound with z+1 number of carbon atoms (higher alkane), respectively.

The *RI* values determined under isothermal

Table 2. Isothermal retention indices for *ortho* and *para* substituted benzylidenemalononitriles on BP-1 column

R_1	R_2	Molecular weight	Melting point (ºC)	R_t (min.)	Hydrocarbons (n+N) and their R_t	RI_I
F	H	172	119	8.232	C-14: 8.028, C-15: 9.283	1417.27
H	H	154	83	9.105	C-14: 8.028, C-15: 9.283	1486.67
CH_3	H	168	104	10.315	C-15: 9.283, C-16: 11.372	1551.94
Cl	H	188	94	10.935	C-15: 9.283, C-16: 11.372	1580.69
Br	H	232	90	13.145	C-16:11.372, C-17: 14.178	1665.60
OCH_3	H	184	84	14.915	C-17: 14.178, C-18: 18.29	1719.90
NO_2	H	199	143	15.985	C-17: 14.178, C-18: 18.29	1747.10
OH	H	170	167	18.49	C-18: 18.29, C-19: 23.455	1804.37
H	F	172	126	8.86	C-14: 8.028, C-15: 9.283	1467.89
H	Br	232	162	11.47	C-16: 11.372, C-17: 14.178	1603.89
H	CH_3	168	134	12.755	C-16: 11.372, C-17: 14.178	1652.04
H	Cl	188	188	16.05	C-17: 14.178, C-18: 18.29	1748.69
H	OCH_3	184	115	17.863	C-17: 14.178, C-18: 18.29	1790.72
H	NO_2	199	159	29.133	C-19:23.455, C-20: 32.367	1967.18

Table 3. Retention indices for ortho and para substituted benzylidenemalononitriles on BP-10 column

R_1	R_2	Molecular weight	Melting point (ºC)	R_t (min.)	Hydrocarbons (n+N) and their R_t	RI_P
F	H	172	119	13.83	C-16: 13.69, C-17: 14.87	1611.86
H	H	154	83	14.92	C-16: 13.69, C-17: 14.87	1704.23
Cl	H	188	94	16.083	C-17: 14.87, C-18: 16.175	1792.95
Br	H	232	90	16.96	C-18: 16.175, C-19: 17.09	1885.8
CH_3	H	168	104	15.873	C-17: 14.87, C-18: 16.175	1776.86
OCH_3	H	184	84	17.775	C-19: 17.09, C-20: 18.13	1967.16
NO_2	H	199	143	19.345	C-20: 18.13, C-22: 20.065	2062.63
H	F	172	126	15.055	C-17: 14.87, C-18: 16.175	1730.06
H	Cl	188	188	17.375	C-19: 17.09, C-20: 18.13	1929.67
H	Br	232	162	18.565	C-20: 18.13, C-22: 20.065	2023.27
H	CH_3	168	134	16.305	C-18: 16.175, C-19: 17.09	1847.22
H	OCH_3	184	115	18.96	C-20: 18.13, C-22: 20.065	2043.48
H	NO_2	199	159	21.175	C-23: 20.95, C-24: 21.805	2326.32
H	OH	170	188	23.25	C-25: 22.67, C-26: 23.71	2555.77

chromatographic conditions were presented in Tables 2 and 4. Like RI_p values, RI_I values were also found to decrease by replacement of the *ortho* substituted chloro group from CS molecule by *-F*, *-H*, $-CH_3$ groups. This replacement led to a decreased interaction of the analyte molecule with the stationary phase and similarly *para* substituted chloro, bromo, methyl, methoxy and nitro analogues resulted in a large increase in the RI_I values because of stronginteractions between the analyte and the stationary phase. Significant changes in RI_I values took place with changes in the stationary phase polarity.

As can be seen from the data in the Tables 1 to 4, the retention indices on the polar stationery phase are higher than those on the non-polar phase. Further the difference in the retention indices value on the polar and non-polar columns (ΔRI_p) was most pronounced for compounds with polar groups such as nitro group in both *ortho* and *para* substituted series. Thus ΔRI_p value could provide information regarding the relative polarities of these compounds (Table 5). The large variations in the values can attribute to the different chemical natures and physical properties of the tear gases studied.

4. RESULTS AND DISCUSSION

Temperature programmed and isothermal retention indices for CS and its analogues were determined on two capillary columns are given in Tables 1 to 4.

The reliability of the retention indices depends on the accurate measurements of retention times and reproducible temperature programming and carrier gas flow-rates. The retention times were measured in up to 0.001 min. increments. The reproducibility during the course of this study was obtained on BP-1 and BP-10 columns. The flow rate of the carrier gas and other chromatographic conditions were maintained constant. The standard deviation of the retention index of any particular compound was less than 0.8 (n=5).

Table 4. Retention indices for *ortho* and *para* substituted benzylidenemalononitriles on BP -10 column

R_1	R_2	Molecular weight	Melting point (°C)	R_t (min.)	Hydrocarbons (n+N) and their R_t	RI_I
F	H	172	119	1.905	C-16: 1.025, C-17: 2.08	1687.58
H	H	154	83	2.255	C-17: 2.08, C-18: 2.58	1737.5
Cl	H	188	94	2.705	C-18: 2.58, C-19: 3.265	1820.1
Br	H	232	90	3.08	C-18: 2.58, C-19: 3.265	1875.22
CH_3	H	168	104	2.24	C-17: 2.08, C-18: 2.58	1734.4
OCH_3	H	184	84	4.048	C-19: 3.265, C-20: 4.24	1982.27
NO_2	H	199	143	6.12	C-20: 4.24, C-22: 7.63	2062.47
H	F	172	126	2.315	C-17: 2.08, C-18: 2.58	1749.69
H	Cl	188	188	3.756	C-19: 3.265, C-20: 4.24	1953.61
H	Br	232	162	5.07	C-20: 4.24, C-22: 7.63	2030.43
H	CH_3	168	134	2.975	C-18: 2.58, C-19: 3.265	1860.5
H	OCH_3	184	115	5.563	C-20: 4.24, C-22: 7.63	2046.22
H	NO_2	199	159	10.97	C-23: 10.61, C-24: 14.725	2310.18
H	OH	170	188	22.162	C-25: 20.567, C-26: 28.81	2522.16

Table 5. Temperature programmed retention indices for ortho and para substituted-benzylidenemalononitriles on BP-1 and BP

R_1	R_2	RI_P		ΔRI_P
		BP-1 column	BP-10 column	
F	H	1409.27	1611.86	202.59
H	H	1472.78	1704.23	231.45
Cl	H	1521.31	1792.95	271.64
Br	H	1670.79	1885.8	215.01
CH_3	H	1624.59	1776.86	152.27
OCH_3	H	1736.39	1967.16	230.77
NO_2	H	1772.78	2062.63	289.85
H	F	1433.14	1730.06	296.92
H	Cl	1650.56	1929.67	279.11
H	Br	1764.8	2023.27	258.47
H	CH_3	1621.35	1847.22	225.87
H	OCH_3	1810.19	2043.48	233.29
H	NO_2	1936.42	2326.32	389.9
H	OH	2020.92	2555.77	534.85

Errors arising from deterioration of the stationary phase can be avoided by use of bonded phases. Application of a small sample size (0.1µl) with nanogram level concentrations of the components helped in avoiding errors that generally arise from overloading of the column. The peaks were sharp and symmetrical even on a polar phase.

5. CONCLUSION

The presence of different functional groups was found to affect the retention indices of CS and its analogues to varying degrees. Replacement of *o–chloro* group from CS molecule resulted in decrease or increase in the retention index value. The retention indices data generated by these studies can be applied for the detection and identification of CS and its analogues in contaminated samples.

ACKNOWLEDGEMENTS

The authors are thankful to Dr M.P. Kaushik, Director, Defence Research & Development Establishment Gwalior for providing the necessary facilities and for useful discussion.

REFERENCES

1. Somani, S.M. (Ed). Chemical Warfare Agents, Academic Press, New York., 1992, pp.443.
2. Gutch, P.K.; Kumar, P.; Suryanarayana, MVS; & Malhotra, R.C. Structure and biological activity relationship of 2-chloro bezylidenemalononitriles, A Riot Control agent. *Def. Sci. J.,* 2005, **55**(4), 447-57.
3. Nigam, A.K.; Suryanarayana, M.V.S.; Gutch, P.K.; Sharma, S.P.; Tamar, L.N.S. & Vijayaraghavan, R. Thermal decomposition studies of riot control agent chloro acetophenon (CN) by Pyrolysis-gas chromatography-mass spectrometry. *J. Hazardous Materials*. 2010, **184**(1-3), 506-14.
4. Koelle, G.B. Pharmacology and toxicology of organo-sphosphates. *In* Clinical and experimental toxicology of organophosphates and carbamates. Ballantyne B, Marrs TC (Eds). Butterworth-Heineman, Oxford, 1992, pp. 33- 37.
5. Gutch, P.K. & Acharya, J. A simple, convenient and effective method for the synthesis of Dibenz (b,f) 1,4-Oxazepines (CR): A new generation riot control agent and its analogues. *Heterocyclic Communication*. 2007**,** **13**(6),393-397.
6. Convention on the Probhition of the development production stockpiling and use of Chemical weapons and on their destruction, technical secretariat of the organization for prohibition of chemical weapons to The Hague, 1977, accessible through internet www.opcw.nl.
7. D'Agostino, P.A. & Provost, L.R. Gas chromatographic retention indices of chemical warfare agents and simulants. *Journal Chromatography*, 1985, **331**(1), 47-54.
8. Gandhe, B.R.; Malhotra, R.C. & Gutch, P.K. Gas chromatographic retention indices of tear gases on capillary columns. *Journal Chromatography*, 1989, **479**(1), 165-169.
9. Jones, G.R.N. CS and its chemical relatives. *Nature*. 1972, **235**, pp.257.
10. Heuck, R. Synthesis of BMNs. *Chem Ber*. 1895, **28**, pp. 2251.
11. Popp, F.D. Synthesis of potential anticancer agents: Pt I, Benzaldehyde nitrogen mustard in the knovenagel reaction. *J. Chem. Soc*., 1960, pp. 5271
12. Shulgin, A.T. US patent 3547966. 1970
13. Malhotra, R.C. & Kumar, P. Chemistry and toxicity of tear gas. *Def. Sci. J.*,1987, **37**(2), 281-96.
14. Van den Dool, H. & Kartz, P.D. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal Chromatography,* 1963, **11(**4),463-471.
15. Mammniner, A. Systematic identification of chemical warfare agents, Ministry for foreign Affair of Finland, Helsinki, 1982, pp-37.
16. Gutch, P.K. Studies on the synthesis, structure activity relationship and degradation of subestituted malonoitriles. Jiwaji University, Gwalior, India,1997. Ph.D.Thesis.
17. Pattsion, J.B. A programmed Introduction to gas-liquid Chromatography. Heyden and Sons, Philadelphia, PA, 1973.
18. Fernandez-Sanchez, E.; Garcia-Dominaguez, J.A.; Menendez, V. & Santiuste, J.M. Programmed-temperature retention indices : A survey of calculation methods. *Journal Chromatography A*, 1990, **498**, 1-9.

Contributors

Dr P.K. Gutch received his PhD (Organic Chemistry) from Jiwaji University, Gwalior, in 1997. He is working as a Scientist in Defence Research and Development Establishment (DRDE), Gwalior. He has published more than 70 research paper in national international journals and has eight patents to his credit. His research interests include: CR, CS, and CN related to defence application. Presently he is working on synthesis of various polymers for SAW –based chemical sensor and decontamination of chemical warfare agents by using organic and polymeric N-Chloro compounds.

Mr R.K. Shrivastava obtained his MSc (Applied Chemistry) from Jiwaji University Gwalior. He is working as Scientist D in DRDE, Gwalior. His areas of research include: Extraction and analysis of toxic chemicals from environmental matrices using analytical techniques like GC and GCMS.