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CHEMISTRY, BIOCHEMISTRY, PHARMACOLOGY, AND TOXICOLOGY OF CS AND SYNTHESIS OF ITS NOVEL ANALOGS

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14. ABSTRACT Of the potential five groups of less-than-lethal riot control and crowd management agents, three groups, namely ω-chloroacetophenones (CN), benzylidene malononitriles (CS-type), and dibenzooxazepines (CR) have found frequent use. Of the three groups, chlorobenzylidene malononitrile (CS) is the most popularly used non-lethal agent in riot control situations and military exercises. With a view to improve its properties and enhance its effectiveness, 15 new CS anlogs incorporating fluorine and fluorine-containing groups have been synthesized using microwave irradiation and novel catalysts. The structures and physical properties and mass spectral characterization of this group of highly potent CS-type agents are described herein.					
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PREFACE

The work was started in May 2003 and completed in September 2004. The experimental details and the spectral data have been described in the monthly/quarterly progress reports. A portion of this work was also presented at the Bio-Defense Symposium organized by the Government of Singapore.

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CONTENTS

1.	INTRODUCTION	7
2.	PHYSIOLOGICAL SYMPTOMS ARISING FROM EXPOSURE TO CS-AGENTS	7
3.	SYNTHESIS OF NOVEL CS-ANALOGS	8
4.	MASS SPECTRAL BREAKDOWN.....	8
5.	PHARMACOLOGY AND TOXICOLOGY OF CS COMPOUNDS.....	13
6.	BIOLOGICAL FATE, MECHANISM OF ACTION AND METABOLISM OF CS	14
7.	MEDICAL APPLICATIONS.....	15
8.	METHODS OF DETECTION OF CS.....	16
9.	PROJECT OBJECTIVE	16
10.	RESULTS AND DISCUSSION	16
10.1	Mass Spectral Fragmentation Mechanism	18
10.2	NMR Data	19
11.	CONCLUSION.....	19
12.	EXPERIMENTAL PART.....	19
12.1	Synthesis of 2-Trifluoromethylbenzylidene Malononitrile (4).....	21
12.2	Condensation of 2-Trifluoromethylbenzaldehyde (3) with Cyanoacetic Acid	21
12.3	Condensation of 2-Trifluoromethylbenzaldehyde (3) with Ethyl Cyanoacetate	21
12.4	Condensation of 2-Trifluoromethylthiobenzaldehyde (7) with Malononitrile.....	22
12.5	Condensation of Pentafluoroacetophenone (27) with Malononitrile.....	22
	LITERATURE CITED	23

FIGURES

1.	Five Groups of Common Crowd Management Agents	7
2.	Symptoms Resulting from Exposure to the CS-Agents.....	8
3.	Metabolism of CS	15
4.	Mass Spectral Fragmentation of Benzylidene Malononitrile	19

TABLES

1.	Synthesis of Novel Potential CS-Agents	9
2.	Mass Spectral Fragmentation of Benzylidene Malononitriles.....	11
3.	Toxicological Studies of 2-Chlorobenzylidene Malononitrile (CS) in Rabbits and Mice	14
4.	Threshold and Concentrations of Non-Lethal Incapacitants	14
5.	Product Distribution with Different Catalysts	17
6.	¹³ C-NMR Chemical Shifts of Substituted Benzylidene Malononitriles	20

CHEMISTRY, BIOCHEMISTRY, PHARMACOLOGY, AND TOXICOLOGY OF CS AND SYNTHESIS OF ITS NOVEL ANALOGS

1. INTRODUCTION

Since the first recorded use of chemicals as weapons of war around 400 B.C., man has used chemicals to manipulate the outcomes of military conflicts. However, since World War I, three groups of organic compounds (**CN**, **CS** and **CR**) have been used to cause temporary incapacitation and have recently found application in crowd control and management situations, though prior to this some of them were employed as chemical warfare (**CW**) agents. The latter activity has now been banned.¹ Of the three groups, two groups - the **CN** and **CR** - seem to have fallen out of grace, and the **CS**-agents have completely replaced the **CN**-agents. The **CS**-agents are currently used as riot control and anti-personnel agents in military training exercises and in testing the protective masks.

The most commonly used crowd control agent is o-chlorobenzylidene malanonitrile (**CS**).² Recently, **CS** was used in copious amounts by law enforcement authorities in Seattle (WA) during the meeting of the World Trade Organization to control and manage disruptive activities of the unruly crowds. The **CS**-agents are internationally used as tear gas agents. Sweden employs it under the trade name **K 62**, in military exercises and testing of the protective masks.

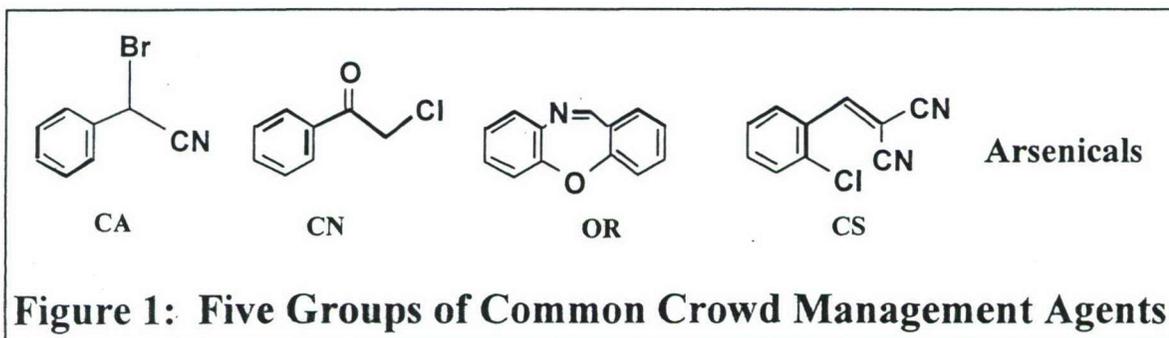


Figure 1: Five Groups of Common Crowd Management Agents

2. PHYSIOLOGICAL SYMPTOMS ARISING FROM EXPOSURE TO CS-AGENTS

In low concentration, the **CS** agents primarily act as irritants and temporary incapacitants by eliciting and inducing various physiological effects including skin irritation, copious flow of tears, running nose, coughing, dizziness, irritation of the respiratory tract, etc.³ In high concentrations, they cause nausea and vomiting. In summary, on exposure to **CS**, one experiences (Figure 2⁴): (1) immediate sensation of skin-burning and skin reddening in warm and moist areas of the body, (2) feeling of pain, (3) watery-eyes and loss of control of the eyelids, (4) irritation and discomfort of the respiratory tract including running nose, tingling sensation in the mouth, throat and upper respiratory tract due to its effect on the nerves of the

mucous membrane, (5) increase in blood pressure and respiration rate in animals exposed to CS aerosols, (6) acidosis and drop in the body temperature from exposures to toxic doses of the CS, (7) production of bradykinin, a vasodilator nona-peptide, in *in vivo* experiments, (8) fall in the pH, (9) sense of disorientation, loss of mental coordination and incapacitation, and (10) tapering off of the effects, in general, within 15 ~ 30 min of the termination of the exposure.

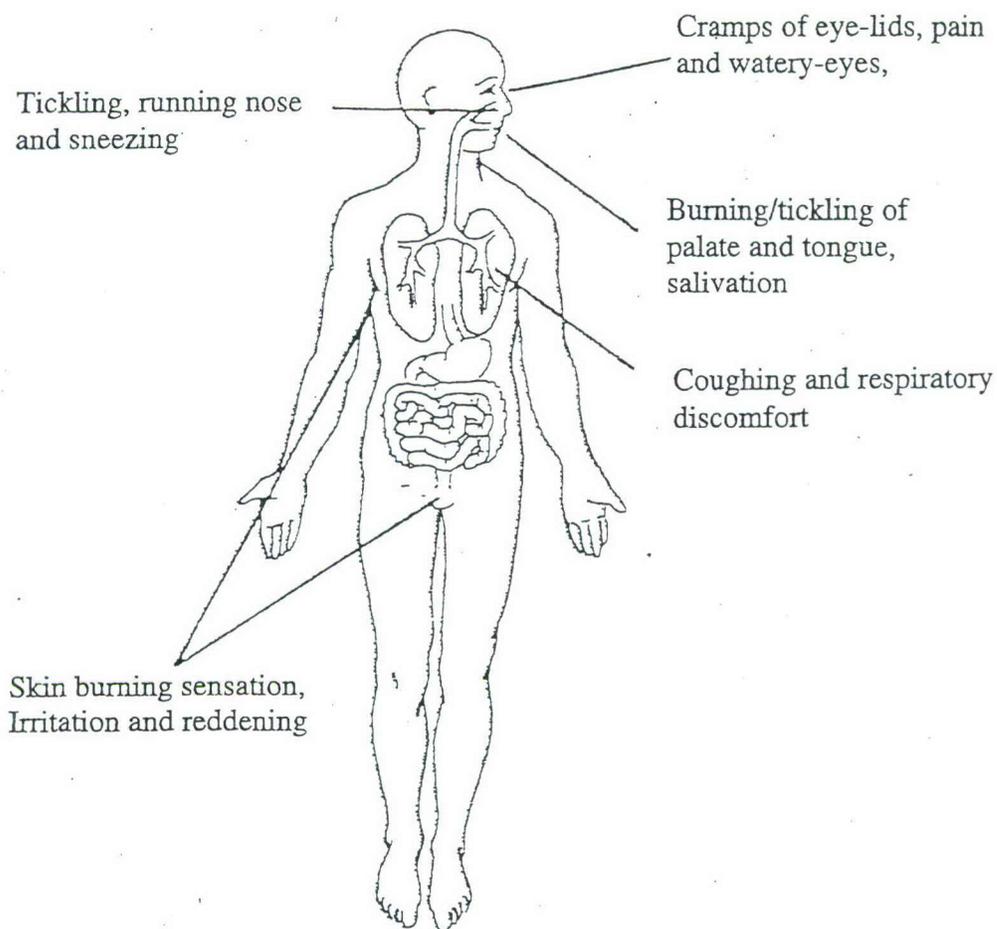


Figure 2: Symptoms Resulting from Exposure to the CS-Agents

3. SYNTHESIS OF NOVEL CS-ANALOGS

See Table 1.

4. MASS SPECTRAL BREAKDOWN

See Table 2.

Table 1: Synthesis of Novel Potential CS-Agents

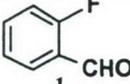
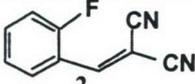
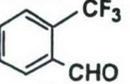
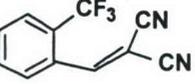
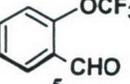
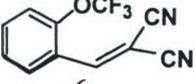
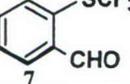
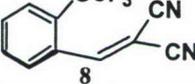
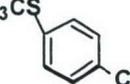
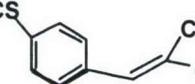
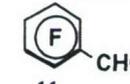
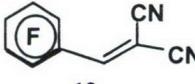
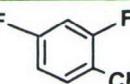
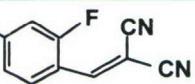
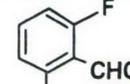
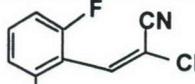
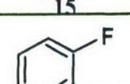
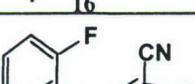
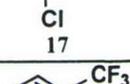
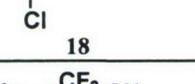
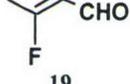
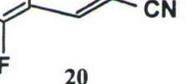
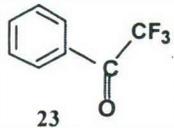
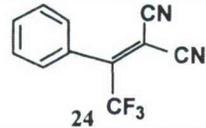
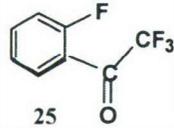
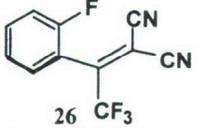
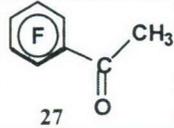
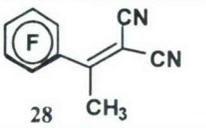
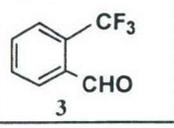
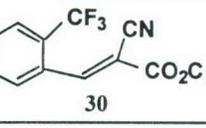
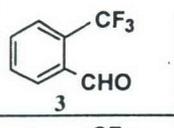
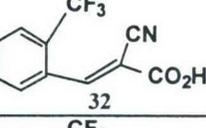
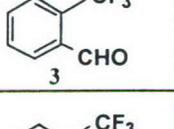
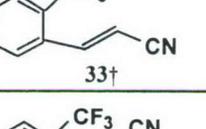
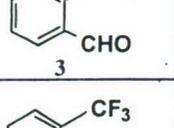
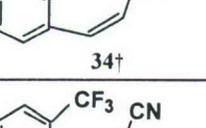
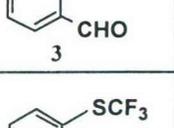
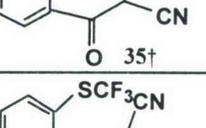
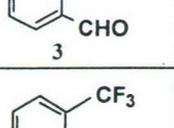
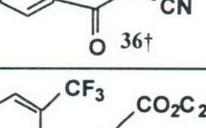
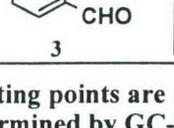
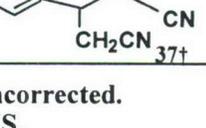
Number	Precursor	Benzylidene malononitrile	m.p. C°	Yield* ⁺	r.t. [§]	NMR δ; (ppm)
1			118 - 19°	86.4%	8.08 min	H = 8.20 F = -111.75
2			48 - 49°	50.0%	7.24 min	H = 8.22 F = -69.26
3			viscous liquid [†]	86.3%	7.54 min	H = 8.18 F = -59.09
4			viscous liquid	87.4%	9.17 min	
5			66 - 67°	88.2%	10.14 min	
6			77 - 78°	98.9%	6.78 min	H = 7.76; F = -74.2; F = -104.21
7			141 - 42°	50.8%	7.69 min	H = 8.83; F = -95.80; F = -106.20
8			77 - 78°	81.2%	7.96 min	H = 8.85; F = -104.15; F = -104.210
9			80 - 81°	95.4%	9.16 min	H = 7.9 F = -103.94
10			92-94°	83.1%	7.06 min	
11			72 - 73°	95.2%	8.99 min	H = 8.01 F = 78.8

Table 1: Synthesis of Novel Potential CS-Agents (continued)

Number	Precursor	Benzylidene malononitrile	m.p.C*	Yield**	r.t.§	NMR
12	 23	 24	viscous liquid	28.5%	6.22 min	
13	 25	 26	92 - 93°	83.1%	6.28min	
14	 27	 28	viscous liquid	37.4%	6.61 min	
15	 3	 30	77 - 78°	955% ***	9.66 min	CH ₃ =1.4 (t); CH ₂ =34 (q); CF ₃ = -59.48
16	 3	 32	165 -166°	73.0%	10.6 min	
17	 3	 33†	*	10.4%	6.06 min	
18	 3	 34†	*	4.4%	6.56 min	
19	 3	 35†	*	10.3%	6.41 min	
20	 3	 36†	*	1.1%	10.67 min	
21	 3	 37†	*	2.3%	11.42 min	

*m.p. melting points are uncorrected.

** Determined by GC-MS.

¶ viscous liquid or low melting solids at room temperature.

§ r.t.= retention time.

*** a mixture of pyridine and 4-dimethylaminopyridine was used as a catalyst.

† side products accompanying the major compound.

Table 2: Mass Spectral Fragmentation of Benzylidene Malononitriles

- (1) 2-Chlorobenzylidene malononitrile (CS, r.t.=9.55 min, 98.7%): M^+ =188; 161 (M - HCN); 153 (M - Cl, 100%); 137 (M - HCCCN); 126 (161 - Cl); 102 (137 - Cl); 99 (126 - HCN); 75 (C_6H_3); 51 (C_4H_3) and 50 (C_3N).
- (2) 2-Fluorobenzylidene malononitrile (2, r.t.=8.08 min, 86.4%): M^+ =172 (99%); 152 (M - HF); 145 (M - HCN, 100%); 121 (M - HCCCN); 118 (145 - HCN); 94 (121 - HCN); 75 (C_6H_3); 63 (C_4HN); 51 (C_4H_3) and 50 (C_3N).
- (3) 2-(Trifluoromethyl)benzylidene malononitrile (4, r.t.=7.24 min, 62.1 %): M^+ =222 (100%); 203 (M - F); 195 (M - HCN); 183 (203 - HF); 176 (195 - F); 171 (M - HCCCN); 153 (M - CF_3); 145 (171 - CN); 126 (153 - HCN); 107 (126 - F); 99 (126 - HCN); 88 (107 - F); 75 (C_6H_3); 51 (C_4H_3 or C_3HN) and 50 (CF_2).
- (4) 2-(Trifluoromethoxy)benzylidene malononitrile (6, r.t.=7.54 min, 86.3 %): M^+ =238 (100%); 219 (M - F); 211 (M - HCN); 172 (219 - HF - HCN); 169 (M - CF_3); 153 (172 - F); 143 (169 - CN); 114 [$C_4H_2C(CN)_2$]; 75 (C_6H_3); 69 (CF_3); and 51 (C_3HN or C_4H_3).
- (5) 2-(Trifluoromethylthio)benzylidene malononitrile (8, r.t.=9.17 min, 87.4 %): M^+ =254; 235 (M - F); 215 (235 - HF); 208 (235 - HCN); 185 (M - CF_3 , 100%); 158 (185 - HCN); 153 (M - SCF_3); 126 (153 - HCN); 114 [$C_4H_2C(CN)_2$]; 75 (C_6H_3); 69 (CF_3); 63 (CSF) and 51 (C_4H_3).
- (6) 4-(Trifluoromethylthio)benzylidene malononitrile (10, r.t.=10.14 min, 88.2 %): M^+ =254 (100 %); 235 (M - F); 215 (235 - HF); 208 (235 - HCN); 185 (M - CF_3 , 97%); 158 (185 - HCN); 153 (M - SCF_3); 114 [$C_4H_2C(CN)_2$]; 100 (C_8H_4); 75 (C_6H_3); 69 (CF_3); 63 (CSF) and 50 (CF_2).
- (7) Pentafluorobenzylidene malononitrile (12, r.t.=6.78 min, 98.9 %): M^+ =244 (100%); 225 (M - F); 218 (M - CN); 199 (225 - CN); 193 (M - HCCCN); ; 168 (C_6HF_5); 148 (168 - HF); 130 (C_6F_3); 124 (175 - HCCCN); 93 (C_3F_3) and 69 (CF_3).
- (8) 2, 4-Difluorobenzylidene malononitrile (14, r.t.=7.69 min, 50.8%): M^+ =190 (100%); 169 (M - HF - H); 163 (M - HCN); 139 (M - HCCN); 124 (C_6F_2N); 101 ($C_5H_3F_2$); 88 (C_5N_2); 75 (C_6H_3); 63 (C_4HN) and 50 (C_3N);
- (9) 2, 6-Difluorobenzylidene malononitrile (16, r.t.=7.96 min, 81.1%): M^+ =190 (100%); 171 (M - F); 163 (M - HCN); 151 (171 - HF); 139 (M - HCCCN); 125 (C_4HNF_2); 112 ($C_6H_2F_2$); 93 (C_5NF); 75 (C_6H_3); 63 (C_4HN) and 50 (C_3N);
- (10) 2-Chloro-6-fluorobenzylidene malononitrile (18, r.t.=9.16 min, 95.4%): M^+ =206 [208 = ^{37}Cl]; 179 [M - $CH=C(CN)_2$]; 171 (M - Cl, 100%); 151 (171 - HF); 144 (179 - Cl); 124 (144 - HF); 118 (144 - CN); 93 (M - C_2H_2 - HCCCN); 85 (C_4H_2Cl); 75 (C_6H_3) and 50 (C_3N).
- (11) 2-Fluoro-6-trifluoromethylbenzylidene malononitrile (20, r.t.=7.06 min, 82.1 %): M^+ = 260 (not seen); 240 (M - HF, 100%); 221 (240 - F); 213 (240 - HCN); 194 (221 - HCN); 190 (240 - CF_2); 171 (M - CF_3); 144 (213 - CF_3); 125 (144 - F); 107 (C_6H_2NF); 99 (125 - C_2H_2); 88 ($C_4H_2F_2$); 75 (C_6H_3) and 57 (C_3H_2F).
- (12) 2, 3-(Difluoromethylenedioxy)benzylidene malononitrile (22, r.t.=8.99 min, 95.4 %): M^+ =234 (100%); 215 (M - F); 168 (M - F_2O_2); 171 (M - Cl, 100%); 151 (171 - HF); 144 (179 - Cl); 140 (168 - HCN - H); 113 (140 - HCN); 101 ($C_4H_2FO_2$), 75 (C_6H_3 ;) and 63 (C_5H_3 or (CO_2F)).

Table 2: Mass Spectral Fragmentation (continued)

- (13) Benzylidene α -(trifluoromethyl)malononitrile (24, r.t.=6.22 min, 28.5 %): M^+ =222 (100%); 202 (M - HF); 195 (M - HCN); 183 (202 - F); 172 (M - HCCCN); 153 (M - CF₃); 126 (153 - HCN); 103 (C₆H₅CN); 88 (C₅N₂); 77 (C₆H₅); 69 (CF₃) and 51 (C₃H₃).
- (14) 2-Fluorobenzylidene α -(trifluoromethyl)malononitrile (26): M^+ =260 (not seen); 240 (M - HF, 100%); 221 (240 - F); 213 (240 - HCN); 194 (221 - HCN); 190 (240 - CF₂); 171 (240 - CF₃); 144 (213 - CF₃); 125 (144 - F); 99 (125 - C₂H₂); 75 (C₆H₃) and 57 (C₃H₂F).
- (15) Pentafluorobenzylidene α -(methyl)malononitrile (28, r.t.=6.61 min, 37.4 %): M^+ =258 (100%); 243 (M - CH₃); 238 (M - HF); 231 (M - HCN); 218 (238 - HF); 212 (231 - F); 193 (212 - F or M - HCCCN); 181 (C₆F₅CH₂); 168 (C₆H₅H); 161 (181 - HF); 148 (168 - HF); 143 (C₆F₃N₂); 117 (143 - CN) and 93 (C₃F₃).
- (16) 2-Trifluoromethyl α -carbethoxycinnamonitrile (30, r.t.=9.73 min, 95.5 %): M^+ =269; 250 (M - F); 240 (M - C₂H₅); 224 (M - OC₂H₅); 200 (M - Cf₃); 196 (M - CO₂C₂H₅); 176 (196 - HF); 172 (CF₃C₆H₄C₂H₃, 100%); 151 172 - H - HF); 126 (C₉H₅N); 99 (C₈H₃); 76(C₆H₄) and 75 (C₆H₃).
- (17) 2-Trifluoromethyl β -cyanocinnamic acid (32, r.t.=6.41 min, 73.4 %): M^+ =241; 224 (M - OH); 197 (M - CO₂); 176 (197 - F); 172 (M - CF₃, 100%); 169 (M - CO₂H - HCN); 128 (192 - CN - OH); 116 (C₇H₃NO); 89 (C₆H₃N); 75 (C₆H₃), 69 (CF₃) and 50 (CF₂).
- (18) trans-2-Trifluoromethylcinnamonitrile (33, r.t.=6.81 min, 10.4 %): M^+ =197 (100%); 177 (M - HF); 176 (177 - H); 170 (M - HCN); 158 (177 - F); 151 (151 - F); 147 (M - CF₂); 128 (149 - F); 120 (147 - HCN); 101 (128 - HCN); 75 (C₆H₃); 69 (CF₃) and 50 (CF₂).
- (19) cis-2-Trifluoromethylcinnamonitrile (34, r.t.=6.95min, 4.3 %): M^+ =197 (100%). The rest of the fragmentation is identical with that of its trans-isomer.
- (20) 2-Trifluoromethylbenzylmalanionitrile (35, r.t.=6.41 min, 10.8 %): M^+ =238; 218 (M - HF); 210 (M - H - HCN); 190 (210 -HF); 173 [M - CH(CN)₂, 100%]; 151 (190 - CHCN); 145 [M - COCH(CN)₂]; 125 (145 - HF); 95 [COCH(CN)₂]; 75 (C₆H₃); 51 (C₃H₄) and 50 (CF₂).
- (21) 2-Trifluoromethylthiobenzylmalanionitrile (36, r.t.=10.67 min, 10.3 %): M^+ =270;201 (M - CF₃); 185 [M - CH(CN)₂]; 173 (201 - HCN - H); 158 (185 - HN); 146 (173 - HCN, 100%); 126 (C₆H₄C₂CN); 114 (146 - S); 69 (CF₃) and 51 (C₄H₃).
- (22) Ethyl (2-tifluoromethylphenyl α -cyanomethyl) cyanoacetate (37, r.t.=10.42 min, 2.3 %): M^+ =310; 281 (M - C₂H₅); 265 (M - OC₂H₅); 245 (265 - HF); 237 (M - CO₂C₂H₅); 217 (237 - HF); 198 (M - CHCH₂CO₂C₂H₅, 98%); 178 (198 - HF, 100%); 156 (198 - CH₂CN); 151 [(NCCHCN(CO₂H₅)); 128 (198 - HCF₃); 101 (C₆H₃CN); 75 (C₅H₃) and 69 (CF₃).

5. PHARMACOLOGY AND TOXICOLOGY OF CS COMPOUNDS

The median concentration for respiratory effects is stated to be 12~20 mg/m³, whereas for eye effects it was found to be 1~5 mg/m³.⁴ The onset time for maximum effects is 20~60 sec and the duration of time is 5~10 min after the cessation of exposure.⁵ The parent compound (CS) exhibits LD₅₀ in rats 28 mg/kg, i.v. rats 48 mg/kg and i.p. LC₅₀ in rats 88 and 480mg/min/m³.⁶ Comparative toxicological studies of CS and CN have been described.^{7,8} Its toxic effects have been investigated.⁹ In a study of its acute toxicity, no abnormalities in EKG, respiratory function, blood biochemistry and cell constitution were observed.¹⁰ On the other hand, the observed decrease in the respiratory rate was found to be proportional to the concentration of CS.¹¹

The CS-aerosol has been reported to cause lachrymation, sternutation, sensory irritation of the upper respiratory tract, skin-sensitization, etc.^{12,13} The on-set time is very rapid.^{6,14} Although several toxicological studies have been conducted, it is rather difficult to accurately describe the mechanisms and effects arising from exposures to the CS vapors. One reason for this is that the *in vivo* half-life of the CS has been observed to be < 30 sec. In fact, it takes that much time to collect blood samples from the experimental animals for analysis. The *in vitro* half-life in blood has been reported to be 4.5 ~ 8.5 sec.¹⁵

Using ¹⁴C-labeled CS, it has been conclusively demonstrated that CS has no effect on DNA.¹⁶ Also, the ¹⁴C-labeling was not incorporated into the DNA to a significant extent. This meant that, unlike mustard, the CS-agents do not act as alkylating agents of the DNA. However, the ¹⁴C-labeling was detected in the proteins; suggesting that either the parent CS or its metabolite(s) react and/or form some sort of binding with proteins. Although the exact nature of this binding has not as yet been completely characterized, it is reasonable to assume that this could be the Schiff base type linkage formed between the free amino group of the protein and the benzaldehyde moiety resulting from the biological breakdown of the CS. The observations of von Doniker and coworkers are supported from the report that CS does not exhibit any mutagenicity.¹⁷ The CS compounds appear to get rapidly hydrolyzed in water to aromatic aldehydes and malononitrile.^{18,19} Because of its exceptional reactivity, malononitrile has not been isolated and identified as such.

In a comparative toxicological study of CN and CS, it was found that neither showed any carcinogenic effects and CS did not cause any permanent damage to the eye, skin, or respiratory system.¹⁵⁻²⁰ The CS is reported to have a lower effective dose value, higher LD₅₀ value and larger safety margin than CN. No dose-response relationship was observed in inhalation studies conducted in mice, rats, and guinea pigs. Ocular administration of 0.5 mL of a 10% solution of CS in dichloromethane produced immediate conjunctivitis, which persisted for 30 ~ 50 min and caused no permanent injury to the eyes. Within 30 sec of exposure, lachrymation and salivation occurred.

Within a few minutes after the cessation of exposure, the effects disappeared. It is important to remember that to date, no carcinogenic effects have been demonstrated from exposures to CS. Because it is considerably less toxic, it certainly has a distinct advantage over CA, CN, CR, and arsenicals. "The gross signs of CS intoxication are lachrimation, salivation,

lethargy and dyspnea”.²⁰ Punte and co-workers have described a toxicological profile of the CS (Table 3) and the results arising from an exposure to CS aerosol.²⁰ Table 4 shows the threshold concentrations of CN, CS, and CR compounds required to produce the desired physiological symptoms.

Modes of Administration	Studies	LD ₅₀ -values
Subcutaneous (mice)	Cumulative	800 mg/ kg
Intravenous and Subcutaneous (rabbits)	Acute	8 mg/ kg

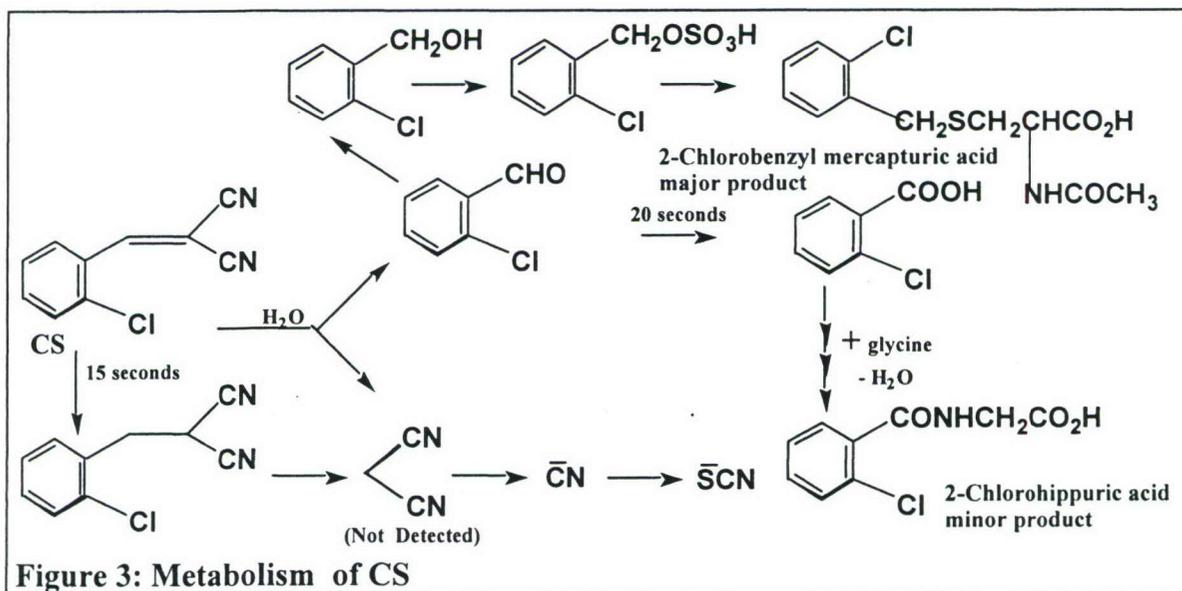
	CN	CS	CR
[TC ₍₅₀₎] (eyes)	0.3	0.004	0.004
[TC ₍₅₀₎] (respiration)	0.4	0.023	0.002
[IC ₍₅₀₎] values	20.5	3.6	0.7
* opcw.nl/chemhaz/tear.htm			

6. BIOLOGICAL FATE, MECHANISM OF ACTION AND METABOLISM OF CS

The CS has been stated to be environmentally non-persistent, which means it is rapidly metabolized and broken down.⁶ However, its *in vivo* metabolic fate seems to depend on its mode of entry into an animal. The fate of the aerosol administered CS is somewhat erratic. When administered intravenously, its metabolism is smooth and is found to rapidly bind with the plasma proteins to form CS-protein complexes.²⁰ Its breakdown begins as soon as it enters the system. After 50 sec, it can be detected in the blood, which happens to be the most active metabolic site of CS, rather than any specific tissue.²⁰ The plasma bound CS may be antigenic.²¹ Inhalation and dermal absorption are the only processes through which the CS can enter into the body. The effects of inhalation are rapid and manifest within a very short time. The manifestation of the effects of dermal absorption takes some time. First, the CS has to be absorbed through the skin and then must reach the nervous system before it can exert its effects.

Lethal and non-lethal chemical agents bring about their effects by interfering with the transmission of nerve impulses either via blocking the transmission or by over flooding the neural system with too many messages to transmit and hence confusing the neural system. This process takes time.

Figure 3 describes the various steps involved in the metabolism of the CS.²² Two pathways have been advanced to explain the metabolism of CS. The first pathway involves the reduction of the exocyclic double bond to form benzyl malononitrile. The second pathway consists of the retro-Knoevenagel type reaction to regenerate the aromatic aldehyde derivative. The benzyl malononitrile, thus formed, has been stated to give rise to malononitrile, which immediately breaks down to CN ion, and in turn is converted into thiocyanate. The aromatic aldehyde formed in the second pathway has two options open to it. The first option is for the aromatic aldehyde to become oxidized to benzoic acid. The latter then yields hippuric acid by reacting with glycine. Second option, the aldehyde becomes reduced to benzyl alcohol, which forms mercapturic acid derivative via the sulfonate ester intermediate. The 2-Chlorobenzylmalononitrile has been identified as a metabolite of CS.²³ Thiocyanate has also been detected after intravenous administration of CS.¹⁴ This lends credence to the proposed pathways. The CS has been observed to react with the thiol moiety at neutral pH.²⁴ Very little or no work has been reported with purified enzyme systems. The CS has been reported to affect the activity of a host of enzymes and also to suppress the activity of esterases.²⁵



7. MEDICAL APPLICATIONS

Although positive results were obtained in treating malignant tumors in animals with CS,^{26,27} studies with human patients suffering from advanced sarcoma and carcinoma were found to be not encouraging.²⁸ "No severe reactions were observed. There were no acute effects on pulse, respiration, temperature, blood pressure, or electrocardiographic patterns. In none of the patients was there an observable effect upon primary tumor or its metastases.

No toxic effects were seen and none of the patients derived subjective or objective clinical improvement".²⁹

8. METHODS OF DETECTION OF CS

Several methods have been developed and described for the detection of CS.³⁰ Benzofurane oxide is reported to give a distinct violet color with CS. A bioassay screening test has been reported,³¹ which essentially consists of applying the sample to the skin and measuring the activity of non-specific esterases using a computer generated image analysis procedure. A quick and dirty method of thin layer chromatographic³² and gas chromatographic techniques^{30,33} have been used in the detection of these compounds.

9. PROJECT OBJECTIVE

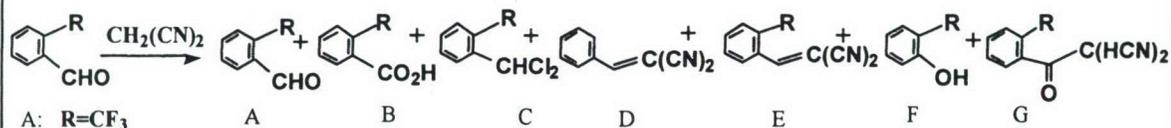
The primary objective of this project was to synthesize environmentally benign, safe, and biologically more potent CS analogs. To this end, the synthesis of a novel group of CS-agents incorporating fluorine and fluorine containing groups has been accomplished under microwave irradiation using new catalysts. This report describes the synthesis and physical properties of a highly potent group of new CS-type compounds (Table 1) and their mass spectral fragmentation behavior (Table 2).

10. RESULTS AND DISCUSSION

Recently benzylidene malonitriles have attracted considerable attention for use as non-lethal crowd control and law enforcement chemical agents. Alkylidenation of the carbonyl compounds via the classical Knoevenagel condensation is of general and wide application for creating a carbon-carbon double bond. In general, this reaction is catalyzed by bases or ammonium salts³⁴. Recently a variety of catalysts such as TiCl₄,^{35,36} CdI₂,³⁷ alumina,^{38,39} zeolites,^{40,41} silylated-amines,⁴² silica,^{43,44} silica functionalized amines⁴⁵ and Al₃PO₄-Al₂O₃ complex⁴⁶ have found application in the Knoevenagel reaction. Solvent free microwave reactions have also been employed for the synthesis of these compounds.⁴⁷ Instead of the carbonyl precursors, imines have been used and found to give similar results.⁴⁸ Even amino acids have been stated to catalyze this condensation.⁴⁹ The Knoevenagel condensation has been carried out diastereoselectively^{50,51} and enantioselectively.^{52,53} Dilithio N-methanesulfinyl-p-toluidine,⁵⁴ tosylmethylisocyanide⁵⁵, α -methoxyvinyl lithium,⁵⁶ lithiated allylic carbamate,⁵⁷ and lithium bis(ethylenedioxyboron)methide⁵⁸ have been employed as catalysts in the modified version of this reaction. Recently, antimony based *in situ* generated catalysts have been used to successfully alkylidenate steroidal ketones.⁵⁹ The reaction has been shown to occur in the presence of LiBr.⁶⁰ From the above narrative, it seems that almost any reagent can catalyze the Knoevenagel reaction. Two mechanisms have been advanced to explain and rationalize the formation of the reaction products.³⁴ Based on the observation that organic bases catalyze the reaction, Hann and Lapworth suggested that the organic bases generate carbanions from the reactive methylene group, which subsequently reacts with the carbonyl compounds to

furnish β -hydroxyl compounds,³⁴ which then undergo facile dehydration during the course of the reaction to yield the end products.

Table 5: Product Distribution with Different Catalysts



	CH ₂ (CN) ₂	A	B	C	D	E	F	G
Activated alumina-bead powder	11.4%	27.8%	1.5%	0.3%*		49.0%		
Europium (III) fluoride	2.2%	3.7%	2.9%	0.7%		90.0%		
Hexafluoroammonium aluminate**	5.9%	19.5%				62.0%	0.6%	10.8%
Piperidine		37.0%	4.0%		0.4%	56.6%		
No catalyst		81.6%	0.4%			8.0%		
Pyridine		1.5%		1.2%	6.7%	88.4%		

* impurity present in the starting material, o-(trifluoromethyl)benzaldehyde.

** Two additional compounds were characterized from this reaction product, namely 2-(trifluoromethyl)-phenol (0.6%) and 2-(trifluoromethyl)benzoyl malononitrile (10.8%).

With a view to developing synthetic methodology, microwave assisted synthesis of benzylidene malononitriles in the neat phase has been explored using new catalysts such as europium (III) fluoride, hexafluoroammonium aluminate, freshly activated alumina-pellet (3 mm) powder, and 1,2,2,6,6-pentamethyl-piperidine. The mass spectral fragmentation and nuclear magnetic resonance (NMR) characteristics of 15 compounds thus synthesized are given in Tables 2, 3, and 6. What is unique about the present work is the characterization of 2-(trifluoromethyl)- and 2-(trifluoromethylthio)benzoyl malononitriles. This suggests that initially formed β -hydroxy intermediates get oxidized during the course of the reaction to furnish the above cited benzoyl malononitriles. To the best of our information, this has not been so far observed in the Knoevenagel reaction. Of the 15 compounds, only one compound, namely 6 (Table 1) has a slightly higher retention time than that of CS. The remaining 14 compounds should, in principle, possess lower vapor pressure than compared to that of CS. The microwave process described herein possesses a distinct advantage. The reactions can be carried without the use of any solvent and thus, avoid the generation and accumulation of hazardous laboratory waste. In addition, the reaction times are dramatically reduced, and the formation of unwanted side-products minimized.

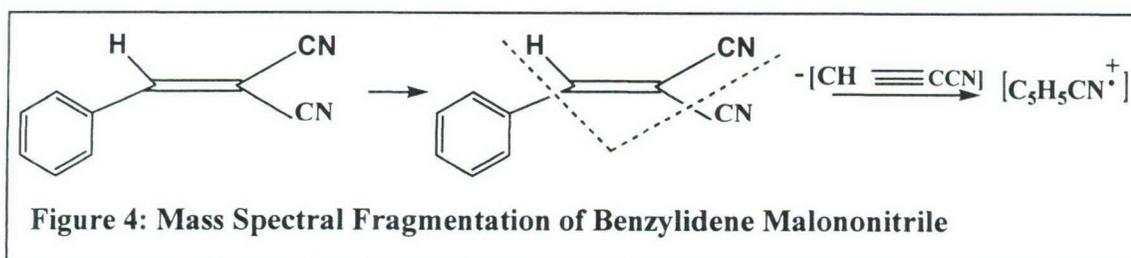
Four new catalysts, [hexafluoroammoniumaluminate, europium (III) fluoride, pentamethylpiperidine, and freshly activated alumina-pellet powder 3 mm] were used in the exploratory work with 2-trifluoromethylbenzaldehyde and malononitrile as reactants. However, in the latter work, only freshly activated alumina-pellet powder was used as a catalyst to avoid the formation of the side-products. In the EuF₃ catalyzed reaction of

2-fluoromethylbenzaldehyde with malononitrile, the presence of 2-trifluoromethylbenzoyl malononitrile (**35**, 0.2%) was detected by gas chromatography mass spectrometry (GC-MS). The identity of the compound was confirmed by the presence of the $[\text{COCH}(\text{CN})_2]$ ion ($m/e=51$) in its mass spectrum. Also, the characterization of 2-trifluoromethylbenzoyl malononitrile (**36**, 10.3%) in the Knoevenagel reaction product formed with hexafluoroammoniumaluminate as catalyst suggests that the initially formed β -hydroxy derivative had undergone *in situ* oxidation.⁶¹ The origin of 2-trifluorophenol is not yet known with certainty, although it could have been formed by the Baeyer-Villiger type oxidation of the substrate during the course of the reaction.

The routine work-up and chromatography over silica of the reaction product of the condensation of 2-trifluoromethylbenzaldehyde with ethyl cyanoacetate in the presence of pyridine and 4-dimethylaminopyridine furnished *trans*-2-trifluoromethylcinnamitrile (1.1%) and ethyl 3-(2-trifluoromethylphenyl)-3-(cyanomethyl)-2-cyanoacetate (**37**, 2.3%) along with the expected product (**30**, see Table 1). The formation of **37** must have involved the Michael-type base catalyzed addition of ethyl cyanoacetate to the cinnamitrile derivative, also formed during the reaction (see Table 1). This inference is supported by the observation that acrylonitrile derivatives are known to serve as substrates in the formation of the carbon-carbon bond during the stepwise Michael type addition reaction.⁶² The characterization of ethyl 3-(2-trifluoromethylphenyl)-3-(cyanomethyl)-2-cyanoacetate as one of the products of the Knoevenagel reaction of 2-trifluoromethylbenzaldehyde with ethyl cyanoacetate was somewhat unexpected, although its genesis can be traced to the Michael type addition of ethyl cyanoacetate to *trans*-2-trifluoromethylcinnamitrile in the presence of basic catalysts. The latter precursor was generated during the course of the reaction. This inference was confirmed by the GC-MS characterization of 2-trifluoromethylcinnamitrile as one of the products of the same reaction. Additional support comes from the recent observation that the ultrasound catalyzed addition of reactive methylene containing compounds to acrylonitriles furnishes the β -adducts.⁶³

10.1 Mass Spectral Fragmentation Mechanism.

Mass spectral fragmentation behavior of benzylidene malononitriles has been discussed briefly.⁶⁴ The primary breakdown process from the parent ion appears to be the loss of hydrogen cyanide (HCN). Also seen is the elimination of propargyl nitrile (HCCCN) moiety from the parent ion generated from benzylidene malononitrile to yield $\text{C}_6\text{H}_5\text{CN}$ radical cation ($m/e=103$) in a one-step process (Figure 4). New bond formation reactions during the electronic bombardment have been observed.⁶⁵ A similar fragmentation behavior is observed during the mass spectral breakdown of the compounds described in this report. The loss of HCN and (HCCCN) fragments ($m/e=27$ and 51) was characteristically observed (Figure 4 and Table 2). The facile loss of F from the molecular ions was also seen in most of the compounds.



10.2 NMR Data.

The NMR spectra of the benzylidene malononitrile have been described.⁶⁶ The chemical shift of the α -proton of the CS-type compounds appears to vary from 7.8 to 8.8 ppm, depending on the nature and position of the substituent on the aromatic ring. Thus, the signal due to ^1H (α -proton of CS) appears at 8.51 ppm in DMSO,⁶⁶ while Mesilaakso observed it at 8.26 ppm in CDCl_3 .⁶⁷ The α -proton has been shown to couple with the ring-hydrogens,⁶⁷ and its chemical shifts depend on the solvents and the nature of substituents on the aromatic ring.⁶⁷ The ^{13}C -signal due to $\text{C}(\text{CN})_2$ was reported to be at 8.52 ppm. The NMR data described herein is similar to this value except in one case, namely that of entry # 6. In view of the reported correlation between the substituent constants and MNR absorption, the above stated changes in the chemical shifts appear to be reasonable and acceptable.⁶⁵ Table 1 gives ^1H and ^{19}F chemical shifts, while Table 6 describes the ^{13}C -NMR data.

11. CONCLUSION

The new CS-analogs are expected to be more potent than CS. This observation is based on the following considerations. First, fluorine is biologically more potent than chlorine. Second, the presence of fluorine makes the new compounds more volatile than CS, and hence, they should all possess lower vapor pressure than that of CS.⁶⁸ This inference stands supported by the fact that they all exhibit lower gas chromatographic retention times than the CS does (Table 1). Because the carbon-fluorine bond is one of the strongest bonds, unlike CS, which contains chlorine, the new compounds ought to be environmentally benign. The presence of fluorine in the new compounds permits their *in vivo* biological screening with PET (Positron Emission Tomography). Thus, the new compounds possess several distinct advantages over CS.

12. EXPERIMENTAL PART

The organofluorine compounds used as substrates in this work were procured from SynQuest Fluorochemicals, Oakwood Research Chemicals and JRD Fluorochemicals, Ltd. (England). Fluorine-containing aldehydes are highly irritating compounds. Care and caution should be exercised to avoid direct exposure to eyes and skin. The alumina pellets (3 mm, Aldrich Chemical Company) were activated by heating at 120 - 130 °C in a heated vacuum oven, cooled to room temperature and freshly ground powder was used immediately. Stoichiometric amounts of the respective reagents were mixed in glass vials or 5 mL ground joint round bottom flasks and stoppered, vigorously shaken on a vibro-mixer and heated in the desktop kitchen Panasonic microwave oven for specified periods of time. The reaction mixture was allowed to come to ambient temperature, taken up in dichloromethane, and filtered over cotton-wool, then first analyzed by gas chromatography and finally was subjected to the GC-MS analysis.

Table 6: ^{13}C -NMR Chemical Shifts of Substituted Benzylidene malononitriles

Entry 1*: CF (163.33); C (ring) CH=C (CN)₂ (126.30); CN (119.45); CN (119.29); CH=C (CN)₂ (159.21); CH=C (CN)₂ (81.89).

Entry 2: CF₃ (210.15); C (ring) CH=C(CN)₂ (132.08); CN (113.13); CN (111.4); CH=C (CN)₂ (156.69); CH=C (CN)₂ (85.62).

Entry 3: OCF₃ (120.62); C (ring)CH=C(CN)₂ (132.51); CN (112.51); CN (113.85); CH=C (CN)₂ (153.62); CH=C (CN)₂ (86.05).

Entry 4: SCF₃ (117.14); C (ring)CH=C(CN)₂ (132.08); CN (117.20); CN (117.28); CH=C (CN)₂ (164.25); CH=C (CN)₂ (78.29).

Entry 6: ; CN (111.95); CN (116.24); CH=C (CN)₂ (142.95); CH=C (CN)₂ (93.82).

Entry 7: CF (161.4); CF (162.0); CN (113.56); CN (112.2); CH=C (CN)₂ (159.03); CH=C (CN)₂ (78.5).

Entry 8: CN (113.56); CN (111.21); CH=C (CN)₂ (159.03); CH=C (CN)₂ (88.1).

Entry 11: CF₂ (111.85); CN (112.84); CN (114.831); CH=C (CN)₂ (149.45); CH=C (CN)₂ (86.78).

Entry 12: CF (136.21); CCl (130.60); CN (112.22); CN (112.31); CH=C (CN)₂ (155.98); CH=C (CN)₂ (88.1).

CS: C (ring)CH=C(CN)₂ (130.6); CCl (136.22); CN (113.13); CN (111.82); CH=C (CN)₂ (155.9); CH=C (CN)₂ (85.6).

* Refers to the serial number in **Table 1**.

Mass spectra were obtained using a Finnigan TSQ-7000 GC/MS/MS equipped with a 30 m x 0.25 mm. i.d. Rtx-5 ms capillary column (Restek, Bellefonte, PA). The conditions on the TSQ-7000 were: oven temperature 60-270 °C at 15 °C/min, injection temperature 220 °C, interface temperature 250 °C, source temperature 150 °C, electron energy 70 eV (EI) or 200 eV (CI), emission current 400 μA (EI) or 300 μA (CI), and scan time 0.7 sec. Data was obtained in the electron ionization mode (range 45-450 da) and chemical ionization mode (mass range 60-450 da). Ultrahigh purity methane was used as the CI agent gas with a source pressure of 3.5 Torr (TSQ-7100). Routine GC analyses were accomplished with a Varian 3400 GC equipped with a J and W Scientific (Folsom, CA) 30 m x 0.53 mm i.d. DB-5 column. Routine GC analyses were accomplished with a Varian 3400 GC equipped with a J and W Scientific 30 m x 0.53 mm i.d. DB-5 column (J and W Scientific, Folsom, CA). The NMR spectra (^1H and ^{13}C) were recorded in CDCl₃ with TMS as the internal standard on a Varian VXR-400S spectrometer at 100 MHz and 376 MHz, respectively. The external reference for ^{19}F was CCl₃F. The melting points were recorded using a Thomas Hoover Capillary melting point apparatus and are uncorrected.

A total of 15 fluorine containing novel CS-compounds were synthesized using the general procedure (cf. entries 1 through 14, Table 1). In addition, 2 substituted cinnamic acid derivatives, 2 substituted benzoyl malononitriles, and 2 cinnamonitriles have been identified.

Because our primary objective was to synthesize novel analogs of CS, the above mentioned side-products were not isolated.

12.1 Synthesis of 2-Trifluoromethylbenzylidene Malononitrile (4) (General Procedure for Activated Alumina-Bead Powder Catalyzed Preparation).

A mixture of stoichiometric amounts of 2-trifluoromethylbenzaldehyde (**3**, 43 mg) and malononitrile (23 mg) and catalytic amount alumina oxide catalyst (20 mg) in a small vial was exposed to microwave radiation for 2.5 min. The reaction mixture was cooled to room temperature, treated with a small amount of dichloromethane, filtered to remove the catalyst and analyzed by GC and then by GC-MS. The above reaction was repeated using different catalysts such as europium (III) fluoride, hexafluoroammonium aluminate and 1,2,2,6,6-pentamethylpiperidine. A similar reaction was carried out under identical conditions except that no catalyst was used in the condensation. Less than 8% of expected product was obtained and the starting material constituted the rest. Table 5 describes the formation and distribution of the various compounds.

12.2 Condensation of 2-Trifluoromethylbenzaldehyde (3) with Cyanoacetic Acid.

Pyridine (0.2 mL) was added to a mixture of 2-trifluoromethylbenzaldehyde (**3**, 0.348 g, 2 mmol) and cyanoacetic acid (0.170 g, 2 mmol), and the whole was shaken with the aid of a vibromixture for 2 min and initially exposed to microwave irradiation for 2 min. Then it was heated for another 2 min. The cooled mixture was processed as usual and the product on GC-MS analysis was found to contain (1) trans-2-trifluoromethylcinnamonnitrile (**33**, 16.4%); cis-2-trifluoromethylcinnamonnitrile (**34**, 4.3%) and β -(2-trifluoromethylphenyl)- α -(cyano) acrylic acid (**32**, 73.0%) along with 2-trifluoromethylbenzaldehyde (**3**, 1.5%) and 2-trifluoromethylbenzalchloride (1.2%). After the solvent was evaporated under reduced pressure, the residue was chromatographed over silica. Elution with petroleum ether removed the last two compounds. The first two compounds were obtained with pet. ether-ether (90:10). The desired compound came out with pet. ether-ether (70:30).

12.3 Condensation of 2-Trifluoromethylbenzaldehyde (3) with Ethyl Cyanoacetate.

Pyridine (0.1 mL, and 4-dimethylaminopyridine (100 mg) was added to a mixture of 2-trifluoromethylbenzaldehyde (**3**, 0.348 g., 2 mmol) and ethyl cyanoacetate (0.226 g., 2 mmol) and was shaken with the aid of a vibromixture for 2 min and heated in a desktop kitchen microwave oven for 4 min. The mixture was cooled to ambient temperature, extracted with methylene chloride (20 mL) and processed as usual. After the solvent evaporated, the residue was chromatographed over silica to yield the product (0.431 g). The GC-MS analysis of the residue showed the presence of three components: (1) trans-2-trifluoromethylcinnamonnitrile (**33**, 1.1%); (2) ethyl β -(2-trifluoromethylphenyl)- α -cyanoacrylate (**30**, 95.5%); and (3) ethyl β -(2-trifluoromethylphenyl)- β -(cyanomethyl)- α -cyanopropionate (**38**, 2.3%).

12.4 Condensation of 2-Trifluoromethylthiobenzaldehyde (7) with Malononitrile.

Europium (III) fluoride (0.100 g) was added to a stoichiometric mixture of 2-trifluoromethylthiobenzaldehyde (**7**, 0.412 g, 2 mmol) and malononitrile (0.132 g, 2 mmol), and the mixture was thoroughly shaken on a vibromixer for 2 ~ 3 min and heated in a desktop microwave oven for 4 min. The mixture after cooling to room temperature was treated with dichloromethane (10 mL), the organic layer was filtered through cotton-wool and the filtrate on GC-MS analysis showed the presence of seven components: (1) benzaldehyde (1.8%); (2) benzyl alcohol (0.9%); (3) 2-trifluoromethylthiobenzaldehyde (**7**, 55.5%); (4) 2-trifluoromethylthiobenzoic acid (0.1%); (5) 2-trifluoromethylthiobenzylidene malononitrile (**8**, 35.1%); (6) 2-trifluoromethylthiobenzoyl malononitrile (**36**, 0.2%); and (7) an unknown compound (0.2%). The benzaldehyde and benzyl alcohol derivatives mentioned above, were not present in the substrate, as shown by the GC-MS analysis of the substrate and hence must have been formed during the course of the reaction. The 2-trifluoromethylthiobenzoic acid obviously resulted from the oxidation of the substrate itself. It is interesting to note the formation of 2-trifluoromethylthiobenzoyl malononitrile (**36**). The last component coming off the column could not be identified as it underwent extensive fragmentation in the ion source.

12.5 Condensation of Pentafluoroacetophenone (27) with Malononitrile.

A stoichiometric mixture of pentafluoroacetophenone (**27**), malononitrile and 1,2,2,6,6-pentamethylpiperidine was reacted as before. The routine processing of the reaction product showed the presence of two compounds: (1) pentafluoroacetophenone (**27**, 62.5%) and (2) pentafluorobenzylidene malononitrile (**28**, 37.4%). The mixture was separated by chromatography over silica.

LITERATURE CITED

1. Renunciation of Certain Uses in War of Chemical Herbicides and Riot Control Agents, Executive Order, 11850 (1975).
2. Corson, B.B.; Stoughton, R.W. Reactions of Alpha Beta Unsaturated Dinitriles. *J. Am. Chem. Soc.* **1928**, *50*, p 2825.
3. Military Chemistry and Chemical Compounds, U. S. Army Field Manual 3-9/U. S. Air Forces Field Manual 355-7, U. S. Government Printing Office, Washington, D.C.
4. Brimblecombe, R.W.; Green, D. M. Green; Muir, A.M. Pharmacology of CS. *Brit. J. Pharmacol.* **1972**, *44*, p 561.
5. Ivarsson, U.; Nilsson, H.; Santesson, J. (eds) A FOA Briefing Book on Chemical Weapons-Threat, Effects and Protection, No. 16, Forsvarets Forskningsanstalt (FOA), Orebro (Sweden) (1992).
6. Ballantyne, B.; Swanston, D.W. The Comparative Acute Mammalian Toxicity of CN and CS. *Arch. Toxicol.* **1978**, *40*, p 75.
7. Gaskins, J.R.; Hehir, R.M.; McCaulley, D.F.; Ligon, E.W. Lachrimating Agents (CS and CN) in Rats and Rabbits: Acute Effects on Mouth, Eye and Skin. *Arch. Environ. Health.* **1972**, *24*, p 449.
8. Elskamp, D.M.W. Toxic Properties of CN and CS. *Sci. Tech. Aerosp. Rep.* 1977, 15 (12) (1977) [cf. Chem. Abstr. 87, p 112594z].
9. Marrs, T.C.; Colgrave, H.F.; Cross, N.L.; Gazzard, M.F.; Brown, R.F.R. A Repeated Dose Study of Toxicity of Inhaled CS Aerosol in Three Species of Laboratory Animals. *Arch. Toxicol.* **1983**, *52*, p183.
10. Beswick, F.W.; Holland, P.; Kemp, K.H. Acute Effects of Exposure to CS and the Development of Tolerance. *Brit. J. Ind. Med.*, **1972**, *29*, p 298.
11. Alarie, Y.; Wakisaka, I; Oka, S. Sensory irritation by SO₂ and CS. *Environ. Physiol. Biochem.*, **1973**, *3*, p 53.
12. Elison, D.H. Handbook of Chemical and Biological Warfare Agents. CRC Press: New York, 2000.
13. Kalman, S.M. Riot Control Agents. *Fed. Proc.*, **1971**, *30*, p 84.

14. Cucinell, S.A.; Swentzel, K.C.; Biskup, R.; Lovre, H.; Stark, W.; Feinsilver, L.; Vocci, F. Biochemical Interactions and Metabolic Fate of Riot Control Agents. *Fed. Proc.*, **30**, **1971**, p 86.
15. Leadbeater, L. Absorption of CS by the Respiratory Tract. *Toxicol. Appl. Pharmacol.* **1973**, **25**, p 101 (1973).
16. Von Daniker, A.; Friederich, U.; Lutz, W.K.; Schlatter, C. Tests for Mutagenicity in Salmonella and Covalent Bonding to DNA and Protein in Rat of the Riot Control Agent CS. *Arch. Toxicol.*, **1981**, **49**, p 15.
17. Wild, D.; Eckhardt, K.; Harnasch, D.; King, M.T. Genotoxicity Study of CS in Salmonella, Drosophila and Mice. *Arch. Toxicol.*, **1983**, **54**, p 167.
18. Patai, S.; Rappoport, Z. Nucleophilic Attack on Carbon-Carbon Double Bonds. *J. Chem. Soc.* **1962**, **392**, p 383.
19. Prichard, R.B.; Lough, C.E.; Reslor, J.B.; Holmes, H.L.; Curries, D.J. The Relative Rates of Reaction of Potassium Cyanide and Water with Substituted Benzalmalononitriles. *Can. J. Chem.* **1967**, **45**, p 775.
20. Pumte, C.L.; Weimer, J.T.; Ballard, T.A.; Wilding, J.L. Toxicological Studies on CS. *Toxicol. Appl. Pharmacol.* **1962**, **4**, p 656.
21. Pinneys, N.S. Contact Dermatitis to CN. *Fed. Proc.* **1971**, **30**, p 96.
22. Boyland, E.; Chasseaud, L.F. Enzyme Catalyzed Conjugations of Glutathione with Unsaturated Compounds, *Biochem. J.* **1967**, **104**, p 95.
23. Leadbeater, L.; Sainsbury, G.L.; Utley, D. 2-Chlorobenzylmalononitrile, a Metabolite Formed from CS. *Toxicol. Appl. Pharmacol.* **1973**, **25**, p 111.
24. Prichard, A.B.; Lough, C.E.; Currie, D.F.; Holmes, H.L. Equilibrium Reaction of n-Butanethiol with Conjugated Heteronoid Compounds. *Can. J. Chem.* **1968**, **46**, p 775, and refs. cited therein.
25. Barry, D.H.; Chasseaud, L.F.; Hunter, B.; Robinson, W.E. Suppression of Non-Specific Esterase Activity in Mouse Skin Sebaceous Gland by CS. *Nature.* **1972**, **240**, p 560.
26. Gal, E.M.; Greenburg, D.M. Growth Retarding Effect of Substituted Malononitriles on transplant Tumors in Mice. *J. Am. Chem. Soc.* **1951**, **73**, p 502.
27. Gal, E.M.; Fung, F.-H.; Greenburg, D.M. Studies in the Biological Action of Malononitriles I: The Effect of Substituted Malononitriles on the Growth of Transplanted Tumor in Mice. *Cancer Res.* **1952**, **12**, p. 565.

28. Tanaka, K. Nucleic Acid Antagonists V: Relationship Between Chemical Structure and Anti-Tumor Activity. cf. *Chem. Abstr.* **1964**, *60*, p 12008h.
29. Petrakis, N.L.; Breman, H.R.; Shimkin, M.B. Substituted Malononitriles in Neoplastic Diseases in Man. *Cancer, Res.*, *12*, p 573.
30. Haddadin, M.; Khalidi, U.; Turjuman, N.; Chougessian, R. Sensitive Test for the Detection of Malononitriles and its Derivatives. *Anal. Chem.*, *46*, p 2072.
31. Barry, D.H.; Chasseaud, L.F.; Hunter, B.; Robinson, W.E. Suppression of Non-Specific Esterase Activity in Mouse Skin Sebaceous Gland by CS. *Nature*, **1972**, *240*, p 560.
32. Ludemann, W.D.; Stutz, M.H.; Sass, S. Qualitative Thin-Layer Chromatography of some Irritants. *Anal. Chem.* *41*, p 679.
33. Sass, S.; Fischer, T.L.; Jascot, M.J.; Herban, J. Gas-Liquid Chromatography of Some Irritants. *Anal. Chem.* **1971**, *43*, p 462.
34. Jones, G. Knoevenagel Condensation. *Organic Reactions*, New York: Wiley and Sons, A. Cope (ed). **1967**, *15*, p. 204.
35. Lehnert, W. Knoevenagel Condensation with $TiCl_4$ /base. *Synthesis*. **1974**, p 667.
36. Rao, P.S.; Venkataratnam, R.V. Zinc Chloride as a New Catalyst for Knoevenagel Condensation. *Tetrahedron Lett.* **1991**, *32*, p 5821.
37. Parajatapati, D.; Sandhu, J.S. Cadmium Iodide as a New Catalyst for Knoevenagel Condensation. *J. Chem. Soc. Perkin Trans.* **1993**, *1*, p 739.
38. Texier-Boulett, F.; Foucod A. Knoevenagel Condensation Catalyzed by Aluminium Oxide. *Tetrahedron Lett.* **1982**, *23*, p 4927.
40. Reddy, T.I.; Varema, R.S. Rare-Earth Exchanged Nay Zeolite Promoted Knoevenagel Condensation. *Tetrahedron Lett.*, **1997**, *38*, p 1721.
41. Wang, Q.L.; Yudao, M.; Zuo, B. Knoevenagel Condensation Catalyzed by Zeolite. *Synth. Comm.* **1997**, *27*, p 4107.
42. Subba Rao, Y.U.; Choudary, B.M. Knoevenagel Condensation Catalyzed by New Nontmorillonitesilylpropylethylenediamine. *Synth. Comm.* **1991**, *21*, p 1163.
43. Kim, S.-Y.; Kwon, P.-S.; Kwon, K.-W.; Chung, S.-K.; Chang, Y.-T. Microwave Enhanced Knoevenagel Condensation of Cyanoacetate with Aldehydes. *Synth. Comm.* **1997**, *27*, p 533.

44. Khan, R. H.; Mathur, R. K.; Ghosh, A.C. Tellurium (IV) Tetrachloride Catalyzed Knoevenagel Reaction. *Synth. Comm.* **1996**, *26*, p 683.
45. Angeletti, I.; Canepa, C.; Martinetti, C.; Venturella, P. Silica Gel Functionalized with Amino Groups as a New Catalyst for Knoevenagel Condensation Under Heterogeneous Catalytic Conditions. *Tetrahedron Lett.* **1988**, *29*, p 2261.
46. Calbetio, J.A.; Campelo, J.M.; Garcia, A.; Luna, D.; Marinas, J.M. Knoevenagel Condensation in the Heterogeneous Phase Using $\text{AlPO}_4\text{-Al}_2\text{O}_3$ as a New Catalyst. *J. Org. Chem.* **49**, **1984**, p 5195.
47. Abdallah-El, A. S.; Texier-Boulett, F.; Hamelin, J. Minute Synthesis of Electrophilic Alkenes under Microwave Irradiation. *Synthesis.* **1994**, 258.
48. Nair, V. J.; Tamera, S. Carbon Elongation with 1,5-Diazapenta-Dienium Salts: Reaction with Aralkyl Ketones. *Synthesis.* **1984**, 424.
49. Yamada, Y.; Iguchi, K.; Hosaka, K.; Hagiwara, K. An Improved Synthesis of Ethyl (dicyanomethylene) Propanoate. *Synthesis.* **1974**, 669.
50. Barrett, A.G.M.; Robyr, C.; Spilling, C.D. Dichloropropoxy-Lithium Nitrones as Reagents for Stereoselective Henry Reactions. *J. Org. Chem.* **1989**, *54*, p 1233.
51. Pyne, S.G.; Boche, C.B. Stereoselective Reactions of Lithium and Zinc Tert-Butyl Phenylmethyl Sulfoxide with Carbonyl Compounds and Imines. *J. Org. Chem.* **1989**, *55*, 2663 (1989)
52. Togni, Pastor, S.D. Chiral Cooperativity: The Nature of Diastereoselective and Enantioselective Step in the Gold(I)-Catalyzed Aldol Reaction Utilizing Chiral Ferrocenylamine Ligands. *J. Org. Chem.* **1990**, *55*, p 1649.
53. Sakuraba, H.; Ushiki, S. Highly Enantioselective Addition of (S)-Lithiomethyl 1-Naphthylsulfoxide to Ketones. *Tetrahedron Lett.* **1990**, *31*, p 5349.
54. Niwa, S.; Soai, K. Catalytic Asymmetric Synthesis of Optically Pure Alkynyl Alcohols by Enantioselective Alkynylation of Aldehydes. *J. Chem. Soc.* **1990**, Perkin Trans. **1**, p 937.
55. Bowlus, S.B.; Katzenellebogen, J.A. An Efficient General Synthesis of Sulfinyltoluidides. *Synth. Comm.* **1974**, *4*, p 137.
56. Schollkopf, U. η -Metalated Isocyanides in Organic Syntheses. *Pure Appl. Chem.*, **1979**, *51*, p 1347.

57. Tanaka, K.; Nakai, T.; Ishikawa, N. N, N-Difluorotosyl-oxyvimyllithium: A Novel Effective Reagent for Preparation of α -Ketoacids from Carbonyl Compounds. *Tetrahedron Lett.* **1978**, 4809.
58. Hoppe, D. The Homoaldol Reaction or How to Overcome Problems of Regio- and Stereo-Selectivity. *Angew. Chem. Internatl.* **1984**, 23, p 932.
59. Davis, A.P.; Battarai, K.M.; Antimony-Based 'Forced Knoevenagel' Methodology for the Conversion of Ketones into Alkylidene Malonates. *Tetrahedron.* 1995, 51, p 8033.
60. Prajapati, D.; Lekhok, K.C.; Sandhu, J.S.; Ghosh, A.C. Lithium Bromide As a New Catalyst for Carbon-Carbon Bond Formation in the Solid State. *J. Chem. Soc., Perkin Trans.* **1996**, 1, p 959.
61. Tanaka, M.; Oota, O.; Hiramatsu, H.; Fujiwara, K. The Knoevenagel Reaction of Aldehydes with Carboxy Compounds: Reactions of P-nitrobenz-aldehyde with Active Methine Compounds. *Bull. Chem. Soc. (Japan)* **1988**, 61, p 2473.
62. Bernasconi, C.F. Nucleophilic Addition to Olefins, Kinetic and Mechanism. *Tetrahedron.* 1989, 45, p 4017; and refs. Cited therein; (b) Marshall, D.R.; Thomas, P.J.; Stirling, C.J.M. Elimination and Addition Reactions: Discrimination between Concerted and Stepwise Processes in Activated Elimination Reactions. *J. Chem. Soc. Perkin Trans.* 1977, 2, p 1914.
63. Li, J.-T.; Cui, Y.; Chen, G.-F.; Cheng Z.-L.; Li, T.-S. Michael Addition Catalyzed by Potassium Hydroxide Under Ultra-Sound. *Synth. Comm.* **2003**, 33, p 353.
64. Williams, D.H.; Budzikiewicz, H.; Djerassi, C. Mass Spectrometry of Organic Compounds; Holden-Day Publishers: San Francisco, 1967; pp 417.
65. Williams, D.H.; Cooks, R.G.; Bowie, J.H.; Madson, P.; Schroll, G.; Lawesson, S.-O. Bond-Forming Reactions Occurring in the Fragmentation of Some α,β -unsaturated Esters and Nitriles upon Electron Impact. *Tetrahedron.* **1967**, 23, p 3173.
66. Hassan, S.S.M.; Abdella, J.M.; Nashed, N.E. Characterization and Determination of Benzalmalonitriles Using Infrared, Nuclear Magnetic Resonance and Mass Spectrometry. *Microchim. Acta.* **1984**, 2, p 27.
67. Mesilaakso, M. Analysis of the ^1H and ^{13}C [^1H] NMR Spectral Parameters of the Tear Gases. *Magn. Reson. Chem.* **1996**, 34, p 989.
68. Kirsch, P. Modern Fluoroorganic Chemistry: Synthesis, Reactivity, Applications. Wiley-VCH Verlag, GmbH and Co.: KgaA, Darmstadt Germany, 2004.