Organic Fluorine Compounds. III. Action of Perchloryl Fluoride on Substituted Ethyl Cyanoacetates and Animal Toxicities of the Fluorinated Products[1,2]

HERMAN GERSHON, STEPHEN G. SCHULMAN, AND A. DAVID SPEVACK

Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York 10701

Received January 21, 1967

C-Alkylated ethyl cyanoacetates (I), when treated with perchloryl fluoride in the presence of sodium ethoxide, were fluorinated in the 2 position. Due to the electronegativity of the fluorine atom, ethanol added across the C≡N bond to form imidates (IV). Further treatment of IV with NH_4OH yielded malonamides (VI). In the presence of sodium or potassium in aprotic solvents, perchloryl fluoride caused the expected cyanofluoroacetate (V) to form. On saponification of V with NaOH, the malonamic acids (IV) were obtained, and in the presence of NH_4OH V yielded VI. Liquid ammonia caused V to be converted to the carboxamidoamidine (VII). Acid hydrolysis of IV and V led to the 2-fluoro fatty acids (VIII). Animal toxicities of the fluorinated compounds are discussed, and fluoromalonamide was found to be relatively nontoxic.

In recent years, there has been a decided interest in substituting fluorine for hydrogen in the attempt to prepare antimetabolites. The rationale employed is that, since hydrogen and fluorine are nearly isosteric, the fluoro analog would be expected to have little difficulty in fitting onto active sites of enzymes. However, the strong electronegativity of the fluorine atom would, when strategically placed, be expected to influence the acid–base character of the metabolite analog and thus would exert a profound influence on the equilibrium between the enzyme, substrate, and the respective complex.

One may regard 5-fluorouracil[4] as a successful example of this rationale. The replacement of the hydrogen atom by fluorine caused a marked increase in the acidity of the product, thereby shifting the equilibrium to favor the formation of the enzyme–substrate complex. It is believed that the formation of thymine from uracil and formate is impaired due to the stability of this complex.[5]

We undertook the preparation of two series of compounds, the 2-fluoro fatty acids and the 2-amino-2-fluoro acids, and it was desired to produce both types of compounds from a common intermediate. The C-substituted ethyl cyanoacetates (I) appeared to be suitable starting materials because they could be α-fluorinated and possessed the carboxyl and nitrogen-containing functions. Scheme I summarizes the approach to the fluorinated metabolite analogs. The starting cyano esters (I) were prepared by published methods: Ic–i,[6] Ib,[7] Ij,[8] and Ik.[9]

It was intended to employ the method of Inman, *et al.*,[10] for the preparation of V, where the cyano esters (I) would be fluorinated by means of perchloryl fluoride in ethyl alcohol in the presence of sodium ethoxide. The desired products were not obtained, but instead, series IV resulted. The structures were established by elemental composition, slight basicity of the products, and the infrared spectra which were characterized by peaks at 1668–1670, 1745–1755, and 3300–3330 cm^{-1}. These were attributed to C=NH, C=O (ester), and =NH, respectively.[11] The absence of a peak at 2000–2300 cm^{-1} due to C≡N was also noted. In addition, the nmr spectrum was consistent for IVb.[12] Upon treatment of IV with concentrated NH_4OH, the malonamide (VI) was obtained.

Since imidates are generally formed from nitriles and alcohols under anhydrous conditions in the presence of acid,[13] it was thought that IV was formed at the conclusion of the treatment with perchloryl fluoride, due to the final acidic pH. Ic was fluorinated in dry ethyl alcohol in the presence of 2 equiv of sodium ethoxide by means of slightly less than 1 equiv of perchloryl fluoride. The product was identified by means of gas chromatography and infrared spectrophotometry as a mixture containing Ic and IVc. The α-fluorine atom enhanced the formation of imidates under strongly basic conditions. It appeared that the literature[10b,c] in which fluorinated cyano esters were prepared by means of perchloryl fluoride in alcohol in the presence of sodium ethoxide was in error. The fluorocyano esters (V) could not be prepared under the conditions reported.[14] A recent reinvestigation of the action of perchloryl fluoride on malonic esters by Gershon, *et al.*,[15] revealed that when perchloryl fluoride reacted with active methylene groups in the presence of ethanol, the alcohol took part in the reaction, causing alkylation of the methylene group, presumably due to the formation of ethyl perchlorate,[16] which acted as the alkylating agent. Although no similar study was made on the products of fluorination of the cyanoacetate esters,

(1) This investigation was supported, in part, by Cancer Chemotherapy National Service Center, National Institutes of Health, under Contract No. PH 43-63-579.

(2) Presented before the 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1964.

(3) Part II: H. Gershon and R. Parmegiani, *J. Med. Chem.*, **10**, 186 (1967).

(4) (a) C. Heidelberger, N. K. Chaudhuri, P. Danneberg, D. Mooren, L. Griesbach, R. Duschinsky, R. J. Schnitzer, E. Pleven, and J. Scheiner, *Nature*, **179**, 663 (1957); (b) A. R. Curreri, F. J. Ansfield, F. A. McIver, H. A. Waisman, and C. Heidelberger, *Cancer Res.*, **18**, 478 (1948).

(5) B. R. Baker, *Cancer Chemotherapy Rept.*, **No. 4**, 1 (1959).

(6) E. R. Alexander and A. C. Cope, *J. Am. Chem. Soc.*, **66**, 886 (1944).

(7) M. A. Pollack, *ibid.*, **65**, 1335 (1943).

(8) M. S. Newman and J. L. McPherson, *J. Org. Chem.*, **19**, 1717 (1954).

(9) C. F. Koelsch, *J. Am. Chem. Soc.*, **65**, 2458 (1943).

(10) (a) C. E. Inman, R. E. Oesterling, and E. A. Tyczkowski, *ibid.*, **80**, 6533 (1958); (b) C. E. Inman, R. E. Oesterling, and E. A. Tyczkowski, U. S. Patent 3,030,408 (1962); (c) C. E. Inman, R. E. Oesterling, and E. A. Tyczkowski, U. S. Patent 3,141,040 (1964).

(11) R. M. Silverstein and G. C. Bassler, "Spectrophotometric Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1963.

(12) R. R. Engle, Riker Laboratories, North Ridge, Calif., personal communication.

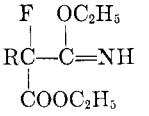
(13) (a) A. Pinner, *Ber.*, **16**, 1643 (1883); (b) *ibid.*, **17**, 171 (1884).

(14) The preparation of fluorodinitriles has been reported by A. D. Josey, U. S. Patent 3,114,763 (1963), who carried out the fluorination of metal salts of dinitriles by perchloryl fluoride in an aprotic solvent, 1,2-dimethoxyethane. Our study had been in progress prior to the publication of this patent.

(15) H. Gershon, J. A. A. Renwick, W. K. Wynn, and R. D. Ascoli, *J. Org. Chem.*, **31**, 916 (1966).

(16) C. E. Inman, E. A. Tyczkowski, R. E. Oesterling, and F. L. Scott, *Experientia*, **14**, 355 (1958).

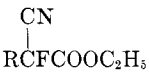
TABLE I
METHYL 2-SUBSTITUTED 2-(1-ETHOXYFORMIMIDOYL)-2-FLUOROACETATES[a]



Compd IV	R	Yield, %	Bp, °C (mm)	$n^{25}D$	Formula	Calcd, % C	H	F	N	Found, % C	H	F	N
b	CH_3	75	74–75 (10)	1.4120	$C_8H_{14}FNO_3$	50.25	7.38	9.94	7.32	50.59	7.45	10.01	7.71
c	C_2H_5	80	84–85 (10)	1.4160	$C_9H_{16}FNO_3$	52.67	7.86	9.26	6.86	53.06	7.72	9.85	7.33
d	C_3H_7	75	97–98 (10)	1.4195	$C_{10}H_{18}FNO_3$	54.78	8.26	8.67	6.43	54.58	8.23	8.21	6.63
e	*i*-C_3H_7	72	91–93 (10)	1.4205	$C_{10}H_{18}FNO_3$	54.78	8.26	8.67	6.43	54.68	8.24	8.26	6.27
f	C_4H_9	77	108–109.5 (10)	1.4230	$C_{11}H_{20}FNO_3$	56.63	8.64	8.14	6.00	57.00	8.58	7.95	6.13
g	*i*-C_4H_9	80	102–104 (10)	1.4235	$C_{11}H_{20}FNO_3$	56.63	8.64	8.14	6.00	56.87	8.18	8.53	6.45
h	*sec*-C_4H_9	80	98–99.5 (10)	1.4206	$C_{11}H_{20}FNO_3$	56.63	8.64	8.14	6.00	57.11	8.90	8.20	5.65
i	$C_6H_5CH_2$	70	105 (0.15)	1.4857	$C_{14}H_{18}FNO_3$	62.90	6.79	7.11	5.24	62.79	6.63	6.75	5.09
j	$C_2H_5OOCH_2CH_2$	90	105 (0.3)	1.4336	$C_{12}H_{20}FNO_5$	51.98	7.27	6.85	5.05	52.51	7.38	6.52	5.50

[a] $\nu_{max}^{C=O}$ 1745–1752 cm^{-1}, $\nu_{max}^{C=NH}$ 1668–1670 cm^{-1} (neat).

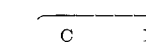
TABLE II
ETHYL 2-SUBSTITUTED 2-CYANO-2-FLUOROACETATES[a]



Compd V	R	Yield, %	Bp, °C (mm)	$n^{25}D$	Formula	Calcd, % C	H	F	N	Found, % C	H	F	N
b	CH_3	35	54–55 (10)	1.3857	$C_6H_8FNO_2$	49.65	5.56	13.09	9.65	49.74	5.72	12.80	9.44
c	C_2H_5	80	67–68 (10)	1.3956	$C_7H_{10}FNO_2$	52.82	6.33	11.94	8.80	53.32	6.62	11.76	8.60
d	C_3H_7	48	79 (10)	1.4016	$C_8H_{12}FNO_2$	55.48	6.98	10.97	8.09	55.50	7.11	11.00	7.99
e	*i*-C_3H_7	80	74 (10)	1.4009	$C_8H_{12}FNO_2$	55.48	6.98	10.97	8.09	56.06	7.04	10.74	7.87
f	C_4H_9	47	90–90.5 (10)	1.4062	$C_9H_{14}FNO_2$	57.74	7.54	10.15	7.48	58.30	7.79	10.12	7.34
g	*i*-C_4H_9	78	83 (10)	1.4060	$C_9H_{14}FNO_2$	57.74	7.54	10.15	7.48	58.22	7.60	10.28	7.37
h	*sec*-C_4H_9	46	86–87 (10)	1.4099	$C_9H_{14}FNO_2$	57.74	7.54	10.15	7.48	57.80	7.84	9.86	7.19
i	$C_6H_5CH_2$	49	123 (4)	1.4832	$C_{12}H_{12}FNO_2$	65.12	5.47	8.59	6.33	64.97	5.63	8.59	6.07
j	$C_2H_5OOCCH_2CH_2$	71	121–121.5 (5)	1.4201	$C_{10}H_{14}FNO_4$	51.94	6.10	8.22	6.06	51.80	6.16	7.97	5.96

[a] $\nu_{max}^{C\equiv N}$ 2235–2335 cm^{-1}, $\nu_{max}^{C=O}$ doublet 1735–1759 and 1770–1780 cm^{-1} (neat).

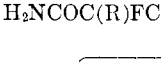
TABLE III
2-SUBSTITUTED 2-FLUOROMALONAMIDES[a]



Compd VI	R	Yield, %	Mp, °C dec[b]	Formula	Calcd, % C	H	F	N	Found, % C	H	F	N
a	CH_3	83	233–234	$C_4H_7FN_2O_2$	35.82	5.26	14.70	20.89	35.45	5.14	14.35	20.91
b	C_2H_5	60	184–185	$C_5H_9FN_2O_2$	40.54	6.12	12.83	18.91	40.88	6.47	13.17	19.10
c	C_3H_7	49	167–168	$C_6H_{11}FN_2O_2$	44.44	6.84	11.72	17.28	44.43	6.92	12.30	17.51
g	*i*-C_4H_9	53	203–204	$C_7H_{13}FN_2O_2$	47.72	7.44	10.78	15.90	48.24	7.28	11.13	16.35
j	$H_2NCOCH_2CH_2$	51	197–198	$C_6H_{10}FN_3O_3$	37.70	5.27	9.94	21.98	37.70	5.34	10.09	22.39

[a] $\nu_{max}^{C=O}$ 1690–1700 cm^{-1} (KBr). [b] All samples were recrystallized from methanol–water (95:5).

TABLE IV
2-SUBSTITUTED 2-FLUOROMALONAMIC ACIDS[a]



Compd IX	R	Yield, %	Mp, °C dec[b]	Neut equiv Calcd	Found	Formula	Calcd, % C	H	F	N	Found, % C	H	F	N
c	C_2H_5	66	142	149	149	$C_5H_8FNO_3$	40.27	5.41	12.74	9.39	40.46	5.27	12.98	9.56
d	C_3H_7	38	154	163	164	$C_6H_{10}FNO_3$	44.17	6.18	11.65	8.59	44.60	6.25	11.80	8.47
e	*i*-C_3H_7	69	159	163	161	$C_6H_{10}FNO_3$	44.17	6.18	11.65	8.59	44.29	6.19	11.74	8.47
f	C_4H_9	58	160	177	177	$C_7H_{12}FNO_3$	47.45	6.83	10.72	7.91	47.66	6.84	10.72	7.98
g	*i*-C_4H_9	60	153	177	177	$C_7H_{12}FNO_3$	47.45	6.83	10.72	7.91	47.61	6.90	10.92	7.76
h	*sec*-C_4H_9	33	145	177	179	$C_7H_{12}FNO_3$	47.45	6.83	10.72	7.91	47.33	6.84	11.09	8.04
i	$C_6H_5CH_2$	66	147	211	211	$C_{10}H_{10}FNO_3$	56.87	4.77	9.00	6.63	56.84	4.71	9.22	6.67

[a] $\nu_{max}^{C=O(CONH_2)}$ 1653–1670 cm^{-1}, $\nu_{max}^{C=O(COOH)}$ 1720–1735 cm^{-1}. [b] All samples were recrystallized from a mixture of acetone–ether–petroleum ether (bp 40–60°).

similar alkylated materials would be expected in the reaction mixtures.

To overcome the disadvantages of a protonated solvent, the fluorination was conducted according to the method of Freeman.[17] Ib was converted to Vb by formation of the potassio salt in the presence of potassium ethoxide, followed by displacement of the ethanol with dry dimethylformamide (DMF) and fluorination with perchloryl fluoride. In addition to the expected elemental composition, the infrared spectrum of the product was characterized by peaks at 1772 and 2250

(17) J. P. Freeman, *J. Am. Chem. Soc.*, **82**, 3869 (1960).

TABLE V

SUMMARY OF ANTICANCER SCREENING DATA[a,b]

Compd	R	SA NTL.[c] mg/kg	SA T/C,[d] %	91 NTL. mg/kg	91 T/C, %	LE NTL. mg/kg	LE T/C, %	KB Slope	KB ED_{50}, mg/ml
IVb	CH_3	500	75	400	129	400	100	−0.63	62
IVc	C_2H_5	500	54	350	72	350	96	−0.77	48
IVd	C_3H_7	500	111	400	140	400	92	−0.83	36
IVe	*i*-C_3H_7	500	118	400	126	400	101	−0.65	66
IVf	C_4H_9	500	140	400	126	400	93	−0.76	60
IVg	*i*-C_4H_9	500	151	400	68	400	107	−0.84	45
IVh	*sec*-C_4H_9	500	81	400	87	400	95	−0.98	36
IVi	$C_2H_5OOCCH_2CH_2$	500	109	400	79	400	93	−1.2	29
IVj	$C_6H_5CH_2$	500	111	400	127	400	96	−1.3	30
		8P		LL		LE		KB	
Vb	CH_3	50	129	50	92	50	87		100
Vc	C_2H_5	200	95	200	95	200	94		100
Vd	C_3H_7	200	103	200	76	100	90		100
Ve	*i*-C_3H_7	400	100	400	89	400	97		100
Vf	C_4H_9	200	95	200	57	200	93		100
Vg	*i*-C_4H_9	200	101	200	97	200	100		100
Vh	*sec*-C_4H_9	400	64	400	101	400	99		100
Vi	$C_2H_5OOCH_2CH_2$	200	70	200	107	200	100		100
Vj	$C_6H_5CH_2$	200	85	200	98	200	96		100
		SA		91 or WM		LE		KB	
VIa	H	500	97	WM 400	90	400	100		100
XIII	F	500	109	WM 400	146	400	96		100
VIb	CH_3	500	134	91 400	127	400	98		100
VIc	C_2H_5	500	107	91 400	200	400	98		100
VId	C_3H_7	250	102	91 200	75	200	104		100
VIg	*i*-C_3H_7	500	127	91 400	83	400	93	−0.74	44
VIi	$H_2NCOCH_2CH_2$	500	95	91 400	117	400	102	−0.60	93
		8P		Misc		LE		KB	
	H[e]			WM 0.2	78	4.0	87		
	F[e]	400	76	SA 500	100	400	95		100
				LS 300	113				
VIIIb	CH_3	50	100	WM 50	113	25	94	−0.22	140
VIIIc	C_2H_5	50	114	WM 25	108	25	103		100
VIIId	C_3H_7			HE 125	66	100	100		100
				FV 100	88				
VIIIe	*i*-C_3H_7	50	101	WM 50	20	25	101		100
				25	41				
				25	75				
				25	63				
VIIIf	C_4H_9	50	91	WM 3.1	16	25	101		100
				3.1	22				
				3.1	34				
				4.6	60				
				3.1	67				
				2.0	74				
				1.3	97				
				6.6	30				
				4.6	33				
VIIIg	*i*-C_4H_9	50	61	DA 25	90	50	88		100
VIIIh	*sec*-C_4H_9	100	132	WM 50	95	50	100		100
				HI 2.5	30				
				2.5	101				
VIIIi	$HOOCCH_2CH_2$	0.62	79	WM 2.5	68	1.5	108		100
VIIIj	$C_6H_5CH_2$	200	66	WM 100	86	125	92	−0.10	23
		HE		Misc		LE		KB	
XII	H			LL 12	32	12	110		100
				12	115				
XVI	F			SA 500	122	125	100		100
				DA 500	95				
IXc	C_2H_5	125	98	FV 100	99	100	98		100
IXd	C_3H_7	125	104	FV 100	123	100	96		100
IXe	*i*-C_3H_7	125	100	FV 100	105	100	111	−1.1	28
IXf	C_4H_7	500	89	FV 400	128	400	95	−1.1	26
IXg	*i*-C_4H_7	500	92	FV 400	85	400	109	−1.2	28
IXh	*sec*-C_4H_7	125	101	FV 100	95	100	98		100
IXj	$C_6H_5CH_2$	500	111	FV 200	84	400	98	−0.9	30

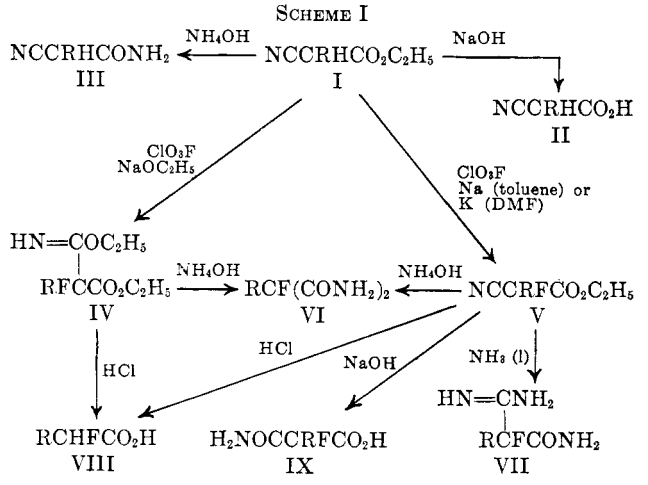
TABLE V (*Continued*)

Misc compd	SA		Misc			L-E		KB
CF_3COOH[e]			FV	100	104	100	93	
			HE	100	94			
			L8	100	112			
$CH_2(COOC_2H_5)_2$[e]	250	66	CA	125	102	250	103	100
X			WM	20	103	0.6	93	
				10	103			
				5.0	102			
				2.5	107			
				0.15	177			
XIV	125	89	WM	100	89	100	91	100

[a] We are indebted to Drs. Howard W. Bond and Harry B. Wood, Jr., Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda, Md. 20014, for making these data available to us. [b] Test tumors employed: SA = Sarcoma 180, 91 = Cloudman melanoma S91, LE = lymphoid leukemia L1210, KB = KB cell culture, 8P = lymphosarcoma P1789, LL = Lewis lung carcinoma, WM = Walker 256 (intramuscular), L8 = lymphoma 8, HE = Hepatoma 129, FV = solid Friend virus leukemia, DA = Dunning ascites leukemia, Hl = human sarcoma HSl, CA = Adenocarcinoma 755. [c] NTL = minimum nontoxic level. [d] T/C = treated tumor/control tumor. [e] Commercially available.

cm^{-1} which were attributed to the α-fluorinated C=O (ester) and C≡N groups, respectively.[11] An improved method for the synthesis of the cyanofluoro esters was based on the formation of the sodio salts of the esters in dry toluene by means of sodium dispersion, and fluorination with perchloryl fluoride. This modification of the fluorination procedure was previously reported by Gershon, *et al.*[3,15,18] The reactions concerning the protonation of the carbon atom of the nitrile group are shown in Scheme I.

SCHEME I



Ia–IIIa, R = H
Ib–VIb, VIIIb, R = CH_3
Ic–IXc, R = C_2H_5
Id–VId, VIIId, IXd, R = C_3H_7
Ie–Ve, VIIIe, IXe, R = *i*-C_3H_7
If–Vf, VIIIf, IXf, R = C_4H_9
Ig–VIg, VIIIg, IXg, R = *i*-C_4H_9
Ih–Vh, VIIIh, IXh, R = *sec*-C_4H_9
Ii–Vi, VIIIi, IXi, R = $C_6H_5CH_2$
Ij, R = $C_2H_5OOCCH_2$
IIj, R = $HOOCCH_2$
IIIj, R = H_2NCOCH_2
Ik, IVk, Vk, R = $C_2H_5OOCCH_2CH_2$
IIk, VIIIk, R = $HOOCCH_2CH_2$
IIIk, VIk, R = $H_2NCOCH_2CH_2$

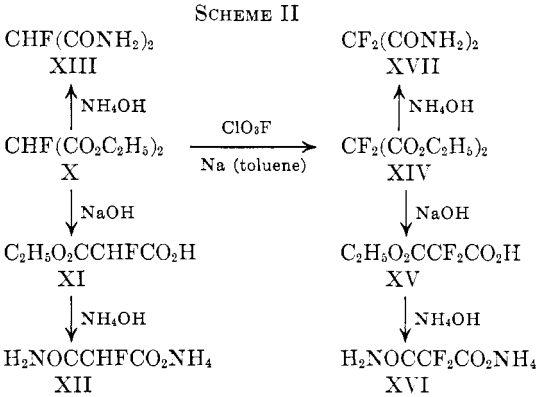
It was evident from these results that the nitrile group was made labile by the strongly electronegative fluorine atom in the α position. This is in agreement with the work of Schaefer, *et al.*,[19] Husted,[20] and Gruber[21] who showed that electron-withdrawing groups α to the nitrile allowed for the formation of imidates and amidines in the presence of basic catalysts.

Hydrolysis of IV and V with concentrated HCl yielded the 2-fluoro fatty acids (VIII). Of the 2-fluoro fatty acids prepared, the following have appeared in the literature: VIIIb,[22,23] VIIIc,[22,24] VIIId,[22] VIIIf,[22] VIIIi,[22,25] and VIIIj.[22,26] A comparison of the antifungal properties of these compounds with nonfluorinated fatty acids was made by Gershon and Parmegiani,[3] who also prepared additional members of the series to 20 carbon atoms. The gas chromatographic separation of VIIIa–h was carried out,[27] and the preparation of the methyl esters of the 2-fluoro fatty acids to 18 carbons and their separation by gas chromatography was also reported.[28]

Advantage was taken of the labile nature of the α-fluoronitriles in order to obtain malonamic acids (IX) which would be suitable for the Hofmann degradation. Compound V was saponified with NaOH to yield IX.

For the preparation of monofluoromalonamic acid (XII), difluoromalonamic acid (XVI), and derivatives, diethyl fluoromalonate (X)[29] and diethyl difluoromalonate[15] were employed as starting materials. These reactions are summarized in Scheme II.

SCHEME II



(18) H. Gershon, S. G. Schulman, and A. D. Spevack, Abstracts, 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1964, p 15K.

(19) (a) F. C. Schaefer and G. A. Peters, *J. Org. Chem.*, **26**, 412 (1961); (b) F. C. Schaefer and A. P. Krapcho, *ibid.*, **27**, 1255 (1962).

(20) D. R. Husted, U. S. Patent 2,676,985 (1954).

(21) W. Gruber, German Patent 1,155,774 (1963).

(22) F. L. M. Pattison, R. L. Buchanan, and F. H. Dean, *Can. J. Chem.*, **43**, 1700 (1965).

(23) E. Gryszkiewicz-Trochimowski and O. Gryszkiewicz-Trochimowski, *Bull. Soc. Chim. France*, 928 (1949); *Chem. Abstr.*, **44**, 3884 (1950).

(24) W. Bockemüller, *Ann.*, **506**, 20 (1933).

(25) E. D. Bergmann and S. Szinai, *J. Chem. Soc.*, 1521 (1956).

(26) L. K. Gottwald, J. E. Ayling, and E. Kun, *J. Biol. Chem.*, **239**, 435 (1964).

(27) H. Gershon and J. A. A. Renwick, *J. Chromatog.*, **20**, 134 (1965).

(28) H. Gershon and J. A. A. Renwick, *ibid.*, in press.

(29) H. Machleidt, *Ann.*, **676**, 66 (1964).

Our experiences with the Hofmann degradation of malonamic acids will be the subject of a subsequent report.

The pertinent data on the fluoroimidates (IV), cyanofluoro esters (V), fluoromalonamides (VI), and fluoromalonamic acids (IX) are contained in Tables I–IV, respectively. All of the compounds were screened by the Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda, Md., against a variety of tumors, and they were found to be generally inactive. The anticancer data on the fluorinated compounds are included in Table V. It should be pointed out that VIIIf showed a significant degree of inhibition against the Walker 256 (intramuscular) tumor in rats, but the results were not consistent.

The toxicity data on the fluorinated derivatives afford a more interesting facet of the biochemical relationships of these compounds. With the discovery of the toxicity of fluoroacetic acid during World War II, research on monofluorinated compounds was stimulated.[30] The high toxicities of ω-fluorofatty acids containing even numbers of carbon atoms, and the low toxicities of those acids containing odd numbers of carbon atoms were explained by Buckle, *et al.*,[31] on the basis that β oxidation of the even numbered ω-fluoro fatty acids produced fluoroacetic acid, and the odd numbered acids yielded β-fluoropropionic acid which was not toxic. Peters[32] presented evidence that fluoroacetic acid is metabolized in the Krebs cycle, *via* a lethal synthesis, to fluorocitric acid, which combines with aconitase causing the cycle to be interrupted. This is accompanied by the accumulation of citric acid. Studies on fluoromalonate in mammals allowed Chari-Bitron[33] to infer that it forms an ester with coenzyme A which is decarboxylated to fluoroacetyl coenzyme A, and then becomes involved in the Krebs cycle. Since the toxic reactions of fluoromalonate and fluoroacetate were qualitatively similar, but the malonate was about one-tenth as toxic as the acetate, the existence of additional metabolic pathways for fluoromalonate was suggested. Bernheim[34] studied the effect of 2,2-difluoromalonamide on the oxidation of organic acids by *Pseudomonas aeruginosa*. It was found that the difluoromalonamide, as well as the free acid, inhibited the oxidation of a number of organic acids of the Krebs cycle, although no inhibition could be demonstrated in sonicates of the cells. The diamides of monofluoromalonic, tetrafluorosuccinic, and hexafluoroglutaric acids were essentially inactive. Pattison, Buchanan, and Dean[22] studied the mammalian toxicities of a series of 2-fluoro fatty acids, and they reported that these compounds showed comparatively low toxicities because they could not undergo β oxidation.

The toxicity data[35] in Table V indicate that most of the fluorinated compounds are not highly toxic. The results on the 2-fluoro fatty acids (VIII) confirm those reported by Pattison, *et al.*,[22] that the fluoro acids from 2-fluoropropionic acid (VIIIb) and above, except 2-fluoroglutaric acid (VIIIi), are relatively nontoxic as compared with fluoroacetic acid. The toxicity of VIIIi is undoubtedly due to the β oxidation of the unfluorinated end of the molecule, and the formation of fluoroacetic acid is the basis for its toxicity. Of the compounds that could potentially yield fluoroacetic acid, fluoromalonamide (VIa), was surprisingly nontoxic, in view of the toxicity of fluoroacetamide.[30] This suggests that VIa cannot be hydrolyzed to a toxic derivative by the animal tissues. The related fluoromalonamic acid (XII) is toxic, and thus it seems that decarboxylation and hydrolysis took place, as expected. VIi, which would be expected to be converted to fluoroglutaric acid and then to fluoroacetic acid, is not toxic, probably due to the formation of a lower fluoromalonamide as the end product. The toxicity of ethyl fluoromalonate (X) was according to expectation.[33]

Experimental Section[36]

2-Cyano-4-methylvaleric Acid (IIg).—Twenty-five grams (0.148 mole) of Ig[6] was saponified with excess NaOH [7.48 g (0.187 mole) of NaOH dissolved in 150 ml of 1:1 aqueous ethanol]. The mixture was stirred at room temperature overnight. Sodium was removed by ion exchange on a column of Amberlite IR-120 (H^+). The effluent was extracted with ether, and the ether was removed by flash evaporation. Water was removed from the aqueous residue by azeotropic distillation with benzene, and the product was distilled, bp 100–105° (0.25 mm), yield 15 g (75%). The analytical sample boiled at 103.5–104°: ν_{max} (C=O) 1720, (C≡N) 2250 cm^{-1}; the neutralization equivalent was 143 (calcd 141).

Anal. Calcd for $C_7H_{11}NO_2$: C, 59.56; H, 7.85; N, 9.92. Found: C, 59.72; H, 8.04; N, 10.05.

2-Cyanosuccinamide (IIIj).—A mixture of 25 g (0.125 mole) of Ij[8] and 100 ml (1.5 moles) of concentrated NH_4OH was kept at 4° with occasional agitation overnight, and then was refrigerated at −10° for 1 week. The product was obtained by filtration and washed with H_2O, and the yield of compound was 9.6 g (58%), mp 163° dec. An analytical sample was obtained by crystallization from aqueous ethanol; mp 164–165.5° dec; ν_{max} (C=O) 1627, 1675, (C≡N) 2250 cm^{-1}.

Anal. Calcd for $C_5H_7N_3O_2$: C, 42.55; H, 5.00; N, 29.78. Found: C, 42.80; H, 5.07; N, 29.49.

2-Cyanoglutaramide (IIIk) was prepared from Ik[9] as above in 83% yield: mp 153–154° (aqueous ethanol); ν_{max} (C=O) 1628, 1660, (C≡N) 2240 cm^{-1}.

Anal. Calcd for $C_6H_9N_3O_2$: C, 46.45; H, 5.85; N, 27.08. Found: C, 46.44; H, 6.12; N, 27.20.

Ethyl 2-(1-Ethoxyformimidoyl)-2-fluoropropionate (IVb).—Sodium (55.0 g, 2.38 g-atom) was dissolved in 1000 ml of anhydrous ethanol, and to the solution was added 300 g (2.38 moles) of ethyl 2-cyanopropionate.[7] The system was purged with dry N_2, and perchloryl fluoride[37] was added to the well-agitated mixture, kept at 10–15° by external cooling. When a heat of reaction was no longer evident, the addition of perchloryl fluoride was interrupted, and the system was again purged with dry N_2. Insoluble salts were removed from the mixture by filtration, and the excess alcohol was flash evaporated. The residue was dissolved in ether, washed free of remaining inorganic salts with H_2O, and freed of ether in the flash evaporator. The product was obtained by distillation; bp 90–92° (20 mm); nmr (neat) τ 9.03 (J = 7 cps), 8.62 (J = 22.5 cps), 2.30.

(30) F. L. M. Pattison, "Toxic Aliphatic Fluorine Compounds," Elsevier Publishing Co., Amsterdam, 1959.

(31) F. J. Buckle, F. L. M. Pattison, and B. C. Saunders, *J. Chem. Soc.*, 1471 (1949).

(32) R. A. Peters, *Proc. Roy. Soc.* (London), **B139**, 143 (1952).

(33) A. Chari-Bitron, *Biochem. Pharmacol.*, **6**, 169 (1961).

(34) F. Bernheim, *Proc. Soc. Exptl. Biol. Med.*, **113**, 411 (1963).

(35) It is conceivable that the toxicities of compounds in tumor-bearing animals may be different from those in healthy animals. However, the data on fluoroacetic acid and difluoroacetic acid are in general agreement with established results.[30] It should also be noted that these data are less precise than LD_{50} determinations.

(36) Melting points were taken in a Mel-Temp melting point apparatus and are uncorrected. Infrared data were obtained with a Perkin-Elmer Model 221 spectrophotometer, and gas chromatography was carried out with an Aerograph Model 204 with a flame-ionization detector to which was attached a Leeds and Northrup Speedomax H recorder, and the column employed was previously described.[15] The procedures described are general.

(37) Perchloryl fluoride was purchased from Pennsalt Chemical Corp., Philadelphia, Pa., along with technical pamphlet DC-1819, "Perchloryl Fluoride," on details of safety and handling.

2-Fluoro-2-methylmalonamide (VIb).—A mixture of 100 ml of concentrated NH_4OH, 25 ml of methanol, and 25 g (0.173 mole) of IVb was kept at −10° with intermittent shaking for 22 days. The crystalline product was obtained by filtration and was dried under vacuum at room temperature. The compound melted at 228–230° dec.

Ethyl 2-Cyano-2-fluoropropionate (Vb).—The potassium salt of ethyl 2-cyanopropionate was prepared by adding 26.0 g (0.205 mole) of the ester to a solution of 7.8 g (0.2 g-atom) of K in 100 ml of anhydrous ethanol. The mixture was brought to dryness under vacuum, and the alcohol was replaced with 200 ml of dry DMF. The DMF was flash evaporated under vacuum and replaced twice. The final residue was kept in the flash evaporator for an additional 1 hr at 100° (15 mm). The dry salt was dissolved in 200 ml of dry DMF, purged with dry N_2 and treated with a rapid stream of perchloryl fluoride. The reaction temperature was maintained at 10–15° by means of an ice bath. When no further heat of reaction was apparent, the system was freed of excess perchloryl fluoride by purging with dry N_2. Inorganic materials were removed by filtration, and the liquid was distilled. The product boiling at 50–55° (10 mm) was collected. This was a mixture of the desired fluoro ester and DMF. The mixture was dissolved in ether and was washed free of DMF with H_2O. Compound Vb was obtained in 40% yield by distillation, bp 55° (10 mm).

Ethyl 2-Cyano-2-fluorobutyrate (Vc).—Sodium dispersion[38] (23 g of Na, 1 g-atom) was suspended in 1000 ml of dry toluene and heated to 50°. To the mixture was added 145 g (1.03 moles) of ethyl 2-cyanobutyrate[6] at such a rate as to keep the temperature of the reaction below 90°. The excess ester was necessary to ensure complete consumption of the Na. The system was purged with dry N_2 and kept at 10–15° by external cooling. A rapid stream of perchloryl fluoride was added, and upon completion of the reaction, as evidenced by cessation of heat evolution, the system was again purged with dry N_2. The inorganic salts were removed by filtration, dissolved in H_2O, and extracted with toluene. The combined toluene layers were washed with H_2O and flash evaporated. The residue was distilled, and the product was collected at 65–70° (10 mm).

2-Fluoro-3-methylbutyric Acid (VIIIe).—A mixture of 43.3 g (0.25 mole) of IVe and 100 ml of concentrated HCl was heated under reflux overnight. The hydrolysate was extracted five times with 100-ml portions of ether, and the ether was removed under a stream of air. The residue was freed of water by azeotropic distillation with benzene and distilled. The yield of product was 24 g (80%), bp 80–83° (10 mm). An analytical sample was obtained by redistillating and collecting a middle fraction, bp 82° (10 mm), mp 41°. The neutralization equivalent was 120 (calcd 120) and $\nu_{max}^{C=O}$ 1720 cm^{-1}.

(38) Purchased from Gray Chemical Co., Gloucester, Mass., as 50% sodium in mineral spirits.

Anal. Calcd for $C_5H_9FO_2$: C, 49.99; H, 7.55; F, 15.82. Found: C, 50.03; H, 7.80; F, 15.55.

2-Fluoro-3-methylvaleric acid (VIIIg) was prepared in the same manner as VIIIe in 80% yield, bp 95.5–96.5° (10 mm), neutralization equivalent 134 (calcd 134), $\nu_{max}^{C=O}$ 1732 cm^{-1}.

Anal. Calcd for $C_6H_{11}FO_2$: C, 53.72; H, 8.26; F, 14.16. Found: C, 53.85; H, 8.15; F, 13.99

2-Fluoro-4-methylvaleric acid (VIIIh) was prepared as VIIIe in 75% yield, bp 96.5–98.5°, neutralization equivalent 134 (calcd 134), $\nu_{max}^{C=O}$ 1725 cm^{-1}

Anal, Calcd for $C_6H_{11}FO_2$: C, 53.72; H, 8.26; F, 14.16. Found: C, 53.42; H, 8.31; F, 14.02.

2-Carboxamido-2-fluorobutyramidine (VIIc).—To 1.41 g (0.01 mole) of Vc in a test tube immersed in a Dry Ice–acetone bath was added 5 ml of liquid ammonia. The mixture was allowed to stand overnight and to come to room temperature with concomitant evaporation of the excess NH_3. The yield of residue was 1.5 g (95%) of VIIc, mp 150–151° dec. An analytical sample was obtained by crystallization from an ethanol–H_2O mixture without change in melting point. The product was basic, and the infrared spectrogram was characterized by a broad band at 1590–1725 cm^{-1}.

Anal. Calcd for $C_5H_{10}FN_3O$: C, 40.81; H, 6.85; F, 12.92; N, 28.56. Found: C, 40.76; H, 6.92; F, 12.88; N, 28.45.

2-Ethyl-2-fluoromalonamic Acid (IXc).—A mixture was prepared containing 6.6 g (0.165 mole) of NaOH, 200 ml of H_2O, and 24 g (0.15 mole) of Vc. After stirring for 2 hr a clear solution resulted, and it was allowed to stand overnight. Sodium was removed by passage through a column of Amberlite IR-120 (H^+), and the effluent was flash evaporated below 40° to apparent dryness. The residue was slurried in ether and the crystalline material was removed by filtration and dried under vacuum. A yield of 10 g of product was obtained which melted at 140° dec.

Ammonium 2-Fluoromalonamate Monohydrate (XII).—To a solution composed of 6.0 g (0.15 mole) of NaOH, 60 ml of H_2O, and 30 ml of ethanol was added 26.7 g (0.15 mole) of diethyl fluoromalonate. The mixture was allowed to stand overnight, and the Na^+ was removed by passage through a column of Amberlite IR-120 (H^+). The effluent was evaporated to near dryness, and the syrupy product was dissolved in methanol made alkaline with NH_4OH and treated with decolorizing carbon, and crystallization was induced by addition of acetone followed by refrigeration. The yield of product was 8.2 g (35%) as the monohydrate, mp 209–211° dec. An analytical sample was obtained by crystallization from a H_2O–methanol–acetone mixture and melted at 209–210° dec.

Anal. Calcd for $C_3H_9FN_2O_4$: C, 23.08; H, 5.81; F, 12.17; N, 17.95. Found: C, 23.30; H, 5.96; F, 12.28; N, 17.56.

Ammonium 2,2-difluoromalonamate monohydrate (XVI) was prepared as above in 47% yield, mp 220–221° dec (methanol–acetone mixture).

Anal. Calcd for $C_3H_8F_2N_2O_4$: C, 20.70; H, 4.63; F, 21.82; N, 16.09. Found: C, 21.01; H, 4.47; F, 22.00; N, 16.12.

Antiinflammatory Dialkylaminoalkylureas

WILLIAM E. COYNE AND JOHN W. CUSIC

Division of Chemical Research, G. D. Searle & Co., Chicago, Illinois 60680

Received February 23, 1967

A series of dialkylaminoalkylureas were synthesized from various tricyclic amines and tested for their antiinflammatory activity.

Previous research in these laboratories has indicated that dialkylaminoalkylureas derived from benzylphenylamines possess antiinflammatory activity.[1] In an attempt to increase the potency of these compounds, ureas derived from various tricyclic amines were prepared. The tricyclic amines used as starting materials were essentially benzylphenylamines bridged at the *ortho* positions of the two aryl groups by O, NR, CH_2, CH_2CH_2, CH=CH, and a single bond. These constitute the 10,11-dihydrodibenz[*b*,*f*][1,4]oxazepines, 10,11-dihydro-5H-dibenzo[*b*,*e*][1,4]diazepines, 5,6-dihydrodibenz[*a*,*d*]azepines, 5,6,11,12-tetrahydrodibenz[*b*,*f*]azocines, 5,6-dihydrodibenz[*b*,*f*]azocines, and the phenanthridines, respectively. Dialkylaminoalkylureas prepared from 10,11-dihydrodibenz[*b*,*f*][1,4]thia-

(1) J. W. Cusic, U. S. Patent 2,681,929 (1950).