Organic Fluorine Compounds. II. Synthesis and Antifungal Properties of 2-Fluoro Fatty Acids[1]

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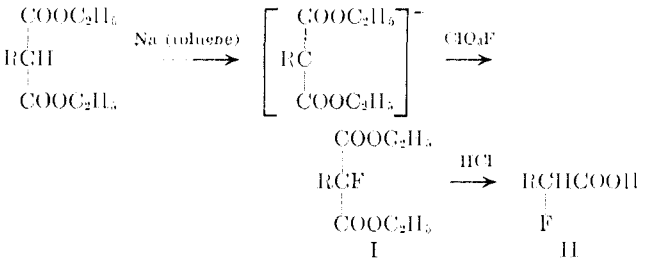
A series of 15 2-fluoro fatty acids to a chain length of 20 carbon atoms was prepared by fluorination of the appropriate alkylmalonic ester by means of perchloryl fluoride followed by acid hydrolysis. The antifungal activity of these compounds was determined in parallel with the corresponding nonfluorinated analogs against four fungi: *Aspergillus niger*, *Trichoderma viride*, *Myrothecium verrucaria*, and *Trichophyton mentagrophytes*. Both series of compounds were about equally active, except that the nonfluorinated fatty acids showed maximal activity at chain lengths of 4–10 carbon atoms, whereas the 2-fluoro fatty acids were most active at chain lengths of 8–14 carbon atoms.

The antifungal activity of fatty acids has been recognized for many years.[2-4] The preceding authors and others[5-8] have demonstrated that the fungitoxicity of these compounds is dependent on chain length, concentration, and pH of the medium. Several materials which act as protective agents against the antimicrobial action of the fatty acids include serum albumin, starch, cholesterol, lecithin, saponin, and charcoal.[9] Although a completely satisfactory explanation of the mode of action of these compounds has not yet been presented, the evidence[10] seems to indicate that growth inhibition is due to alteration in cell permeability.

As a result of our earlier work,[11] the 2-fluoro fatty acids to 2-fluorohexanoic acid were available for further study. A critical examination of the preparation of 2-fluoro fatty acids was reported by Pattison, *et al.*[12] Of the fluorination procedures studied, the method of Inman, *et al.*,[13] in which methylene groups could be fluorinated by perchloryl fluoride in the presence of strong base, was considered least satisfactory, due to difficulties in separating fluorinated from nonfluorinated compounds and due to low over-all yields. A recent reinvestigation of the action of perchloryl fluoride on diethyl malonate by Gershon, *et al.*,[1] revealed that when perchloryl fluoride reacted with active methylene groups in the presence of alcohol, the alcohol took part in the reaction, causing alkylation of the methylene group, presumably due to the formation of alkyl perchlorate, which acted as the alkylating agent. Thus, a number of side products resulted in addition to the desired fluorinated esters. The side reactions were eliminated by conducting the fluorination of the sodio esters, formed by means of sodium dispersion, in dry toluene. In this manner, fluorinated esters were obtained in good yield, which on hydrolysis afforded 2-fluoro fatty acids, with a minimum of purification.

In order to compare the antifungal activity of 2-fluoro fatty acids with the nonfluorinated fatty acids, additional members of the fluoro fatty acid series were prepared by fluorinating the sodio salts of alkylated malonic esters in dry toluene by means of perchloryl fluoride, according to Scheme I.

SCHEME I



a, R = CH_3 — b, R = C_8H_{17}
b, R = C_2H_5 — i, R = C_9H_{19}
c, R = C_3H_7 — j, R = $C_{10}H_{21}$
d, R = C_4H_9 — k, R = $C_{12}H_{25}$
e, R = C_5H_{11} — l, R = $C_{14}H_{29}$
f, R = C_6H_{13} — m, R = $C_{16}H_{33}$
g, R = C_7H_{15} — n, R = $C_{18}H_{37}$

Of the 28 compounds prepared, the fluoromalonic esters Ib–d[12] were previously prepared and characterized, and Ia and Im[12] were prepared but not characterized. In the 2-fluoro fatty acid series, IIa,[11,12,14] IIb,[11,12,15] IIc and IId,[11,12] and IIe, IIf, IIh, and IIm[12] were previously reported. The pertinent data on the fluoromalonic esters (I) and 2-fluoro fatty acids (II) are contained in Tables I and II, respectively, and the infrared spectra of these compounds show that the $\nu_{max}^{C=O}$ for the malonic esters ranges between 1750 and 1765 cm^{-1} and between 1710 and 1740 cm^{-1} for the fluoro fatty acids.

The fluorinated fatty acids, IIa–m and fluoroacetic acid were screened in parallel with the corresponding nonfluorinated fatty acids against three fungi, *Aspergillus niger*, *Trichoderma viride*, and *Myrothecium verrucaria*, in Sabouraud dextrose agar (Difco) at pH 5.6 and pH 4.0. Spores of the various organisms were exposed to three concentrations of each test compound, 10^4, 10^3, and 10^2 μg/ml for 5 days at 28°. Records of

(1) Part I of this series: H. Gershon, J. A. A. Renwick, W. K. Wynn, and R. D'Ascoli, *J. Org. Chem.*, **31**, 916 (1966).
(2) J. F. Clark, *Botan. Gaz.*, **28**, 289 (1899).
(3) A. Kiesel, *Ann. Inst. Pasteur*, **27**, 391 (1913).
(4) O. Wyss, B. J. Ludwig, and R. R. Joiner, *Arch. Biochem.*, **7**, 415 (1945).
(5) K. Kitajima and J. Kawamura, *Bull. Imp. Forestry Expt. Sta., Japan*, **31**, 108 (1931); *Chem. Abstr.*, **26**, 4693 (1932).
(6) S. Tetsumoto, *J. Agr. Chem. Soc. Japan*, **9**, 388 (1933).
(7) S. Tetsumoto, *ibid.*, **9**, 563 (1933).
(8) R. H. Thornton, *New Zealand J. Agr. Res.*, **6**, 469 (1963).
(9) C. Nieman, *Bacteriol. Rev.*, **18**, 147 (1954).
(10) E. Kodicek and A. N. Worden, *Biochem. J.*, **39**, 78 (1945).
(11) H. Gershon, S. G. Sebulman, and A. D. Spevack, Abstracts, 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1964, p 15K.
(12) E. L. M. Pattison, R. L. Buchanan, and F. H. Dean, *Can. J. Chem.*, **43**, 1700 (1965).
(13) C. E. Inman, R. E. Oesterling, and E. A. Tyczkowski, *J. Am. Chem. Soc.*, **80**, 6533 (1958).
(14) E. Gryszkiewicz-Trochimowski and O. Gryszkiewicz-Trochimowski, *Bull. Soc. Chim. France*, 928 (1949); *Chem. Abstr.*, **44**, 3884 (1950).
(15) W. Boekenmüller, *Ann.*, **506**, 20 (1933).

TABLE I

DIETHYL ALKYLFLUOROMALONATES (I)

$RCF(COOC_2H_5)_2$

Compd I	R	Yield, %	Bp (mm), °C	$n^{25}D$	Formula	Calcd, % C	Calcd, % H	Calcd, % F	Found, % C	Found, % H	Found, % F
a[a]	CH_3	84	47 (55)	1.3986	$C_8H_{13}FO_4$	50.00	6.82	9.89	50.44	6.65	9.83
e	C_5H_{11}	75	94–95 (1.1)	1.4169	$C_{12}H_{21}FO_4$	58.05	8.53	7.65	58.32	8.31	7.93
f	C_6H_{13}	70	104–105 (1.15)	1.4199	$C_{13}H_{23}FO_4$	59.52	8.84	7.24	59.88	8.88	7.39
g	C_7H_{15}	64	105 (0.55)	1.4224	$C_{14}H_{25}FO_4$	60.85	9.12	6.88	61.34	8.86	7.09
h	C_8H_{17}	64	114–115 (0.55)	1.4254	$C_{15}H_{27}FO_4$	62.05	9.37	6.54	61.88	9.24	6.49
i	C_9H_{19}	57	122 (0.55)	1.4278	$C_{16}H_{29}FO_4$	63.13	9.60	6.24	63.01	9.94	6.19
j	$C_{10}H_{21}$	71	143 (0.90)	1.4287	$C_{17}H_{31}FO_4$	64.12	9.81	5.97	64.20	9.82	5.95
k	$C_{12}H_{25}$	87	162 (1.1)	1.4328	$C_{19}H_{35}FO_4$	65.86	10.18	5.48	66.06	10.13	5.56
l	$C_{14}H_{29}$	89	164–166 (0.50)	1.4358	$C_{21}H_{39}FO_4$	67.34	10.50	5.07	67.69	10.55	5.37
m[a]	$C_{16}H_{33}$	89	168–169 (0.55)	1.4394	$C_{23}H_{43}FO_4$	68.61	10.77	4.72	68.67	10.62	4.93
n	$C_{18}H_{37}$	89	199 (0.40)[b]		$C_{25}H_{47}FO_4$	69.73	11.00	4.41	69.90	10.87	4.51

[a] Compound mentioned but not characterized in ref 12. [b] Mp 31–32°.

TABLE II

2-FLUORO FATTY ACIDS (II)

RCHFCOOH

Compd II	R	Yield, %	Mp, °C	Formula	Neut equiv Calcd	Neut equiv Found	Calcd, % C	Calcd, % H	Calcd, % F	Found, % C	Found, % H	Found, % F
g[a]	C_7H_{15}	84	52.5–53.5	$C_9H_{17}FO_2$	177	177	61.00	9.67	10.72	61.44	9.44	10.61
i[a]	C_9H_{19}	75	61–62	$C_{11}H_{21}FO_2$	204	203	64.67	10.36	9.30	64.87	10.62	9.20
j[b]	$C_{10}H_{21}$	85	69–70	$C_{12}H_{23}FO_2$	218	218	66.02	10.62	8.70	66.11	10.52	8.56
k[a]	$C_{12}H_{25}$	37	75–76	$C_{14}H_{27}FO_2$	246	246	68.35	11.05	7.71	68.04	11.10	7.53
l[a]	$C_{14}H_{29}$	43	82–83	$C_{16}H_{31}FO_2$	274	275	70.03	11.39	6.92	70.08	11.57	7.03
n[c]	$C_{18}H_{37}$	61	89.5–90.5	$C_{20}H_{39}FO_2$	331	336	72.68	11.89	5.75	72.83	11.61	5.94

[a] Crystallized from acetone–water mixture. [b] Crystallized from benzene. [c] Crystallized from benzene–petroleum ether (bp 40–60°) mixture.

growth or absence of growth were tabulated, and the data were weighted by calculating the antifungal spectrum index which is defined as the total number of levels of inhibition multiplied by the number of organisms inhibited within the defined system. This constant was previously employed and discussed by Gershon and Parmegiani[16] and Gershon, Parmegiani, and Nickerson.[17] The antifungal data on the nonfluorinated and 2-fluorinated fatty acids are summarized in Table III.

The data confirm, in general, that the antifungal activity of the nonfluorinated fatty acids is dependent on chain length, and that activity increases with a decrease in pH of the medium. The same generalizations hold true for the fluorinated fatty acids, except that a longer chain length is required for comparable activity. Whereas, in this test system, the nonfluorinated fatty acids are most active at chain lengths of 4–10 carbon atoms, the fluoro fatty acids are most active at chain lengths of 8–14 carbon atoms. Since the pK_a values of the 2-fluoro fatty acids are generally 2 units lower than those of the nonfluorinated fatty acids, it appears that the pK_a of the fatty acid does not play an important role in its antifungal activity.

To examine the effect of adsorbents on the antifungal activity of these compounds, the pathogenic fungus *Trichopyton mentagrophytes* was employed as the test organism. The medium used was Sabouraud dextrose agar enriched with 10% beef serum (Difco) at pH 5.6 and 7.0. The antifungal results are summarized in Table IV. It was also confirmed that beef serum has a deactivating effect on the nonfluorinated fatty acids as

(16) H. Gershon and R. Parmegiani, *Appl. Microbiol.*, **10**, 348 (1962).

(17) H. Gershon, R. Parmegiani, and W. J. Nickerson, *ibid.*, **10**, 556 (1962).

TABLE III

ANTIFUNGAL ACTIVITY OF FATTY ACIDS AND 2-FLUORO FATTY ACIDS AT pH 5.6 AND pH 4.0

R	Levels of inhib at pH 5.6[a] *A. niger*	*T. viride*	*M. verrucaria*	Antifungal spectrum index[b]	Levels of inhib at pH 4.0[a] *A. niger*	*T. viride*	*M. verrucaria*	Antifungal spectrum index[b]
				RCH_2COOH				
H	1	1	1	9	1	2	2	15
CH_3	1	1	1	9	1	2	2	15
C_2H_5	1	2	2	15	1	3	3	21
C_3H_7	1	2	2	15	2	3	3	24
C_4H_9	1	2	2	15	1	3	3	21
C_5H_{11}	1	2	2	15	2	3	3	24
C_6H_{13}	1	2	2	15	2	3	3	24
C_7H_{15}	2	2	2	18	2	3	3	24
C_8H_{17}	1	3	3	21	0	3	3	12
C_9H_{19}	0	2	3	10	0	3	3	12
$C_{10}H_{21}$	0	0	0	0	0	1	3	1
$C_{12}H_{25}$	0	0	0	0	0	0	0	0
$C_{14}H_{29}$	0	0	0	0	0	0	0	0
$C_{16}H_{33}$	0	0	0	0	0	0	0	0
$C_{18}H_{37}$	0	0	0	0	0	0	0	0
				RCHFCOOH				
H	0	0	0	0	0	0	1	1
CH_3	0	0	0	0	0	1	1	4
C_2H_5	0	0	1	1	0	1	1	4
C_3H_7	0	1	1	4	1	1	2	12
C_4H_9	0	0	1	1	1	1	2	12
C_5H_{11}	0	0	0	0	1	1	2	12
C_6H_{13}	1	1	1	9	1	3	3	21
C_7H_{15}	1	2	2	15	2	3	3	24
C_8H_{17}	1	2	2	15	2	3	3	24
C_9H_{19}	1	3	3	21	3	3	3	27
$C_{10}H_{21}$	1	2	2	15	1	3	3	21
$C_{12}H_{25}$	1	1	1	9	1	2	2	15
$C_{14}H_{29}$	0	0	0	0	0	0	0	0
$C_{16}H_{33}$	0	0	0	0	0	0	0	0
$C_{18}H_{37}$	0	0	0	0	0	0	0	0

[a] Compounds incorporated in test medium at 10^4, 10^3, and 10^2 μg/ml; 3 = inhibition at all levels of compound, 2 = inhibition at two highest levels, 1 = inhibition at highest level only. [b] Antifungal spectrum index = total number of levels of inhibition multiplied by number of organisms inhibited.

TABLE IV

COMPARISON OF ANTIFUNGAL ACTIVITY OF SELECTED 2-FLUORINATED WITH NONFLUORINATED FATTY ACIDS AGAINST *T. mentagrophytes* AT pH 5.6 AND 7.0 IN SABOURAUD DEXTROSE AGAR IN THE PRESENCE AND ABSENCE OF BEEF SERUM AFTER 5 DAYS AT 28°

	Levels of inhib.[a]			
	pH 5.6		pH 7.0	
R	− serum	+ serum	− serum	+ serum
	RCH_2COOH			
C_6H_{13}	3	3	3	3
C_7H_{15}	3	3	3	3
C_8H_{17}	3	3	3	2
C_9H_{19}	3	2	3	2
$C_{10}H_{21}$	2	2	3	2
$C_{12}H_{25}$	..	..	..	..
	$RCHFCOOH$			
C_6H_{13}	.	.	.	.
C_7H_{15}	2	2	2	2
C_8H_{17}	3	2	2	2
C_9H_{19}	3	2	3	2
$C_{10}H_{21}$	3	3	3	2
$C_{12}H_{25}$	3	2	3	2

[a] Compounds incorporated in test medium at 10^3, 10^2, and 10^2 μg/ml: 3 = inhibition at all levels of compound, 2 = inhibition at 2 highest levels, 1 = inhibition at highest level only.

well as on the 2-fluoro fatty acids, and that deactivation is greater at pH 7.0 than at pH 5.6.

It can be said, in general, that the antifungal activity of the 2-fluoro fatty acids parallels that of the nonfluorinated fatty acids.

As systemic antifungal agents, the fatty acids, in spite of their low order of toxicity, have been ineffective. This may be due to their being readily metabolized by the host through the usual fatty acid pathways. They may be esterified to form glycerides and may be degraded to small fragments by β oxidation. Asami, *et al.*,[18] have recently demonstrated that 11-iodo-10-undecenoic acid is esterified, in part, in the rat to a glyceride. Although the effect of fluorine in the 2 position of fatty acids on esterification has not yet been reported, these fatty acids are believed not to undergo β oxidation[12] and, consequently, show comparatively little toxicity. Thus the 2-fluoro fatty acids possess at least one potential advantage over the nonfluorinated analogs which would be useful for systemic antifungal activity.

Experimental Section

Chemical.[19] **Diethyl Dodecylfluoromalonate (Ik).**—Sodium dispersion[23] (9.3 g, 0.405 g-atom of sodium) was suspended in 500 ml of dry toluene and was titrated with 135 g (0.41 mole) of diethyl dodecylmalonate.[20,24] Perchloryl fluoride (41.5 g, 0.405 mole) was added to the well-stirred suspension in a rapid stream, keeping the temperature at 10–15° by means of an ice bath. Upon completion of addition of the gas, the inorganic materials were removed by filtration, and the toluene was flash evaporated under vacuum. The residue was filtered through a fine sintered-glass filter and distilled. The yield of product was 122 g (87%), bp 200–207° (10 mm), $n^{25}D$ 1.4316.

2-Fluorotetradecanoic Acid (IIk). A mixture of 50 g (0.17 mole) of diethyl dodecylfluoromalonate (Ik) and 200 ml of concentrated HCl was heated under reflux overnight. The product was extracted with ether, and the ether was flash evaporated. The residue was dissolved in benzene and freed of water by azeotropic distillation and then taken to dryness by flash evaporation. The yield of fluorinated fatty acid was 45.5 g (97%), mp 73.5–74.5°.

Antifungal Assay. Buffers.[25]—The following buffers were employed: pH 4.0, Sörensen's citrate HCl buffer; pH 5.6, Sörensen's citrate NaOH buffer; pH 7.0, phosphate buffer.

Media. Sabouraud dextrose agar at pH 5.6 was prepared in the appropriate buffer to 60% of its final volume and autoclaved at 15 lb/in.^{2} for 15 min. Test compounds were dissolved or suspended in 3.5 ml of water at such concentrations as to yield 10^3, 10^2, and 10^2 μg/ml when diluted to 10 ml. The mixtures were adjusted to pH 5.6 with 10% NaOH, diluted to 4.0 ml, and sterilized by autoclaving. Six milliliters of medium was mixed with 4 ml of test compound, while keeping the temperature of both between 50–60°. The mixtures were poured in equal portions into three chambers of quadrant Petri plates, and, after hardening, each portion of medium was inoculated with 1 drop of spore suspension containing 6×10^6 spores/ml of the respective organism: *A. niger*, *T. ciride*, and *M. racemosa*. After 5 days at 28°, growth or absence of growth was recorded.

At pH 4.0, Sabouraud dextrose broth (Difco) was dissolved in the buffer, and the dextrose concentration was doubled to bring it to the level of Sabouraud dextrose agar before diluting to 50% of the final liquid volume. An equal volume of a solution of agar (Difco) in water was prepared to contain sufficient agar to yield a 1.5% solution at its final dilution. Test compounds were prepared as previously described. All three liquids were sterilized by autoclaving, and, while still hot, the Sabouraud dextrose broth solution was mixed with the agar solution, and the Petri plates were prepared and treated as above. This procedure was necessary at the low pH because, under these acid conditions, agar will hydrolyze and will not set on cooling.

At pH 7.0, Sabouraud dextrose agar was dissolved in the proper buffer to 60% of its final volume, and where 40% beef serum was used, it was added aseptically to the medium prepared to 50% of its final volume. Petri plates were prepared as described and inoculated with 1 drop of a spore suspension of *T. mentagrophytes* containing 6×10^6 spores/ml and treated as above. This organism was also tested at pH 5.6 in a similar manner.

(18) Y. Asami, H. Kusakabe, A. Eriguchi, K. Amemiya, M. Itabe, A. Ueno, G. Saito, S. Sakai, Y. Morooki, and Y. G. Tanaka, *Riken Gaku Kenkyusho Hokoku*, **41**, 259 (1965); *Chem. Abstr.*, **64**, 18271 (1966).

(19) 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 refractive indices were taken in a Bausch and Lomb, Abbe-3L refractometer. The synthetic procedures are general, and the lower alkylmalonic esters to pentyl were commercially available. Where the alkyl groups were hexyl to octadecyl, the esters were prepared according to the method of Rothstein.[20] Although the tetradecyl,[21] hexadecyl,[22] and octadecyl[23] esters were not reported by Rothstein, the compounds were known.

(20) R. Rothstein, *Bull. Soc. Chim. France*, [5] **2**, 801 (1935).

(21) E. Chargaff, *Ber.*, **65B**, 745 (1932).

(22) G. S. Skinner and A. P. Stuart, *J. Am. Chem. Soc.*, **63**, 2993 (1941).

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

(24) The end point of the titration was determined by cessation of hydrogen evolution, since there was no way to measure the exact quantity of sodium to be transferred. A slight excess of the malonic ester was always employed to ensure complete utilization of the sodium.

(25) W. M. Clark, "The Determination of Hydrogen Ions," 3rd ed, The Williams & Wilkins Co., Baltimore, Md., 1928.