Synthesis of 4,4-difluoro analogs of retinol and retinoic acid

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Abstract Oxidation of retinol at the C-4 position is a major metabolic route to biologically inactive excretory products. Thus, the replacement of hydrogen with fluorine at C-4 might well modify the rate of metabolism, biological activity, and pharmacological activity of vitamin A. 4,4-Difluororetinyl acetate and related analogs were consequently synthesized by the following procedure. Retinoic acid (**1**), upon methylation to methyl retinoate (**2**), was oxidized with manganese dioxide to methyl 4-oxoretinoate (**3**). Methyl 4,4-difluororetinoate (**4**), prepared from methyl 4-oxoretinoate (**3**) by reaction with diethylaminosulfurtrifluoride (DAST), was further converted to 4,4-difluororetinoic acid (**5**), 4,4-difluororetinol (**6**), 4,4-difluororetinyl acetate (**7**), and 4,4-difluororetinyl palmitate (**8**). 4,4-Difluororetinyl acetate was also prepared directly from 4-oxoretinyl acetate (**9**) by reaction with DAST. The purified, often crystalline, analogs were characterized by UV absorption, mass spectrometry, and ^{1}H-NMR and ^{19}F-NMR spectra.—**Barua, A. B., and J. A. Olson,** Synthesis of 4,4-difluoro analogs of retinol and retinoic acid. *J. Lipid Res.* **25:** 304–309.

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Vitamin A is an essential nutrient for growth, vision, and reproduction (1). At high doses vitamin A and its synthetic analogs, the retinoids, are also efficacious in the treatment of many skin disorders (2) and for the reversal and prevention of neoplastic transformations (3–6). Since optimally effective doses of these compounds are often toxic as well, the search has continued for new analogs of vitamin A. In designing some new vitamin A analogs, we have particularly considered the following factors: *1*) substituting fluorine for hydrogen in steroids and other pharmacological agents is often efficacious (7) and has been shown to improve the anti-cancer properties of some retinoids (8, 9); *2*) the analysis of ^{19}F-labeled analogs of vitamin A by nuclear magnetic resonance spectroscopy has recently enhanced our knowledge of the molecular interaction between retinal and opsin (10, 11); and *3*) a major pathway for the metabolic inactivation of retinoids is oxidation at the C-4 position in the trimethylcyclohexene ring (12–14). We reasoned that blocking the C-4 position of vitamin A with fluorine might reduce the rate of its metabolic inactivation and excretion and thereby enhance its pharmacological effectiveness.

In this report we therefore describe the synthesis of all-*trans* 4,4-difluororetinyl acetate (**7**), as well as some related derivatives. The formulas and names of these derivatives are given in **Fig. 1**.

MATERIALS AND METHODS

Chemicals and solvents

All-*trans* retinoic acid was purchased from Sigma Chemical Co. (St. Louis, MO), and precipitated MnO_2 was obtained from the British Drug Houses (Poole, England). Diethylaminosulfurtrifluoride (DAST) was prepared according to Markovskij, Pashinik, and Kirsanov (15) and Middleton (16) or was purchased from the Aldrich Chemical Co. (Milwaukee, WI). Neutral alumina for column chromatography, supplied by J. T. Baker Chemical Co. (Philipsburg, NJ), was deactivated by the addition of water (8%, v/w). Silica gel for dry column chromatography, activity III/30 mm, was obtained from Woelm Pharma (Eschwege, West Germany). All other reagents, solvents, and chemicals were AR grade.

Chromatographic technique

High pressure liquid chromatography (HPLC) was conducted with a Waters Associates U6K injector and 6000A pump equipped with a Perkin-Elmer LC-75 variable wavelength detector and a Hewlett-Packard 3390A integrator. For reverse-phase separations, a C_{18} μBondapak column (0.4 cm × 30 cm) was used with methanol or with methanol–water 4:1 containing 10 mM ammonium acetate as the eluant at a flow rate of 2 ml/min; for straight-phase separation, a Type M9 Partisil 10 column (0.94 cm × 50 cm) was employed with 1–5% ethyl acetate in hexane as the eluant at a flow rate of 4 ml/

Abbreviations: HPLC, high pressure liquid chromatography; DAST, diethylaminosulfurtrifluoride; TLC, thin-layer chromatography; app. t of d, apparent triplet of a doublet in NMR spectra.

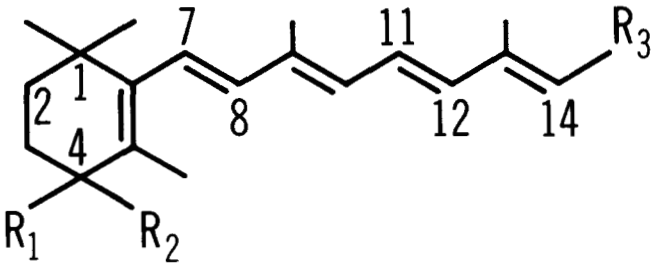

Fig. 1. Analogs of all-*trans* retinol.

No.	R_1R_2	R_3	Name
1	H, H	COOH	Retinoic acid
2	H, H	$COOCH_3$	Methyl retinoate
3	O	$COOCH_3$	Methyl 4-oxoretinoate
4	F, F	$COOCH_3$	Methyl 4,4-difluororetinoate
5	F, F	COOH	4,4-Difluororetinoic acid
6	F, F	CH_2OH	4,4-Difluororetinol
7	F, F	CH_2OCOCH_3	4,4-Difluororetinyl acetate
8	F, F	$CH_2OCO(CH_2)_{14}CH_3$	4,4-Difluororetinyl palmitate
9	O	CH_2OCOCH_3	4-Oxoretinyl acetate
10	H, H	CH_2OCOCH_3	Retinyl acetate
11	O	COOH	4-Oxoretinoic acid
12	O	CH_2OH	4-Oxoretinol

min. Thin-layer chromatography (TLC) was conducted on silica gel plates (0.25 mm × 20 cm × 20 cm) obtained from the Brinkmann Instrument Co. (Westbury, NY) with either hexane–diethyl ether 4:1 or hexane–acetone 2:1 as the developing solvent.

Physicochemical and spectrometric analysis

Ultraviolet spectra were recorded with a Perkin-Elmer model 552 UV-visible recording spectrophotometer. Mass spectra were obtained using a direct inlet probe with a Finnegan model 4000 GC/MS operating in the EI mode at 70 eV. For new compounds, the mass and the relative intensity (in parentheses) only of major and of diagnostic peaks are given. Molecular ions are denoted as M^+. The ^{1}H-NMR and ^{19}F-NMR spectra of vitamin A analogs dissolved in $CDCl_3$, containing either trimethylsilane or $CFCl_3$ as an internal reference, were recorded with a Bruker 300 MHz instrument. The amounts of vitamin A and its difluoro analogs were measured by comparing integrated peak areas of HPLC fractions with those of appropriate standards at a selected wavelength.

EXPERIMENTAL SECTION

Preparation of methyl retinoate (2)

All-*trans* retinoic acid (3 g, 100 mmol) in 75 ml of warm ethyl acetate was treated with anhydrous K_2CO_3 (10 g) and CH_3I (15 ml), refluxed for 2 hr, and then cooled. The ethyl acetate solution was washed thrice with 50 ml of water, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residual orange oil was dissolved in hexane and chromatographed on a column (100 g) of water-deactivated alumina. Methyl retinoate, which was eluted with hexane, was concentrated in vacuo to an orange oil and was crystallized from pentane after storage at −20°C. Yield: 3 g, 96%; UV max (hexane) 354 nm (ε 45,200); MS: m/z 314, M^+ (100), 299 (14), 283 (2), 267 (8), 255 (33), 177 (85), 159 (49), 125 (86), 69 (84); ^{1}H-NMR (**Table 1**).

Preparation of methyl 4-oxoretinoate (3)

A modification of the procedure of Barua and Ghosh (17) was employed. Methyl retinoate (3 g, 9.5 mmol) in 100 ml of CH_2Cl_2–hexane 1:1 was stirred with 30 g (0.34 mol) of precipitated MnO_2 (added in two portions of 15 g within 1 hr) at room temperature for 20–30 hr. The solution was filtered, the precipitate was washed with CH_2Cl_2 until the washings were colorless, and the pooled filtrate was roto-evaporated to dryness. The residual oil was dissolved in hexane and chromatographed on a column of deactivated alumina. Methyl 4-oxoretinoate was eluted with 20% diethyl ether in hexane. When the concentrated product was stored at −20°C in hexane, crystalline methyl 4-oxoretinoate (1.77 g) (mp 93–94°C) was obtained. The average yield of methyl 4-oxoretinoate in five experiments was 50%. The crystalline compound showed the following spectral characteristics: UV max (methanol) 362 nm (ε 57,400) 288 nm (ε 8,700); (hexane) 355,280 nm (**Fig. 2**); MS: m/z 328, M^+, 313 (M^+-15, CH_3), 296 (M^+-32), 269 (M^+-59, $COOCH_3$); ^{1}H-NMR (Table 1).

Preparation of methyl 4,4-difluororetinoate (4)

Methyl 4-oxoretinoate (1.15 g, 3.5 mmol) in the form of an oil was treated with 565 mg (3.5 mmol) of DAST in a closed screw-top Teflon bottle at 50°C for 16–20 hr in the dark. After cooling, diethyl ether and crushed ice were added, and the ether layer was removed, dried over anhydrous Na_2SO_4, and roto-evaporated to dryness. The residue was dissolved in hexane and chromatographed on a column of deactivated alumina (100 g). An isomeric mixture of the difluoro analogs from methyl 4-oxoretinoate was eluted with 5% diethyl ether in hexane. On the basis of an ε of 50,000, cited below, the range of yields of the difluoro ester from methyl 4-oxoretinoate was 5–10%. Unreacted methyl 4-oxoretinoate, (70–80% recovery), which could be retreated with DAST, was eluted with 20% ether in hexane.

The isomeric mixture of difluoro esters in hexane was separated by HPLC on a Partisil column, using 1% ethyl acetate in hexane, into four major and two minor fractions (**Table 2**). After removal of the solvent from each fraction by roto-evaporation, the residual oils were dissolved in minimal volumes of hexane and kept at −20°C. In fraction

TABLE 1. 300 MHz ^{1}H-NMR spectra of all-*trans* and 13-*cis* 4,4-difluororetinoids and related compounds

Compound[a]	Chemical Shift, δ												Coupling Constants, Hz[b]				
	1-CH_3	2-CH_2	3-CH_2	4-CH_2	5-CH_3	7-CH	8-CH	9-CH_3	10-CH	11-CH	12-CH	13-CH_3	14-CH	Other	$J_{2,3}$	$J_{7,8}$	$J_{10,11}$	$J_{11,12}$	$J_{14,15}$
1[c]						6.29	6.14	2.01	6.15	7.03	6.31	2.37	5.79	3.70 (OCH_3)					
2	1.03	1.47	1.62	2.03	1.72	6.29	6.14	2.01	6.15	7.01	6.29	2.37	5.78	3.72 (OCH_3)	*d*	16	11	15	
3	1.17	1.86	2.52		1.86	6.36	6.30	2.04	6.26	6.99	6.36	2.36	5.82	3.72 (OCH_3)	7	16	11	15	
4	1.06	1.66	2.10		1.80	6.20	6.19	2.00	6.20	6.98	6.32	2.36	5.80	3.71 (OCH_3)	*d*	16	11	15	
13-*cis* **4**	1.06	1.66	2.18		1.80	6.22	6.14	2.00	6.30	6.96	7.81	2.08	5.68	3.71 (OCH_3)	*d*	16	11	15	
5	1.06	1.66	2.18		1.80	6.23	6.16	2.00	6.21	7.01	6.35	2.38	5.82		*d*	16	11	15	
13-*cis* **5**	1.06	1.66	2.18		1.80	6.23	6.16	2.00	6.21	7.01	7.78	2.10	5.70		*d*	16	11	15	
7	1.06	1.66	2.10		1.80	6.18	6.11	1.96	6.15	6.63	6.32	1.89	5.64	2.07 ($COCH_3$)	*d*	16	11	15	7
														4.73 (15–CH_2)					
10[d]	1.03				1.70	6.18	6.12	1.95	6.09	6.65	6.27	1.89	5.61	2.05 ($COCH_3$)	*d*	~16	11	15	7
														4.70 (15–CH_2)					
13-*cis* **10**[e]						6.17	6.10	1.95	6.12	6.69	6.59	1.95	5.47	2.05 ($COCH_3$)					
														4.74 (15–CH_2)					
13-*cis* 1[c]						6.29	6.17	2.00	6.27	7.03	7.77	2.10	5.69						

[a] Compounds are all-*trans* isomers unless designated otherwise.
[b] To the nearest whole Hz.
[c] Values of Vetter et al. (22).
[d] Multiplets were not readily resolved.
[e] Values of Patel (21).

Fig. 2. Ultraviolet absorption spectra in hexane of all-*trans* methyl 4,4-difluororetinoate (——); 13-*cis* methyl 4,4-difluororetinoate (-----); 4,4-difluororetinol (–·–·–); and methyl 4-oxoretinoate (–··–··–).

6, all-*trans* methyl 4,4-difluororetinoate crystallized in shining orange-yellow needles (20 mg); mp 84–86°C; UV max (hexane) 340 nm (ε 50,000) (Fig. 2); (methanol) 348 nm: MS: m/z 350, M^+ (10), 330 (41), 310 (9), 291 (15), 251 (38), 193 (37), 177 (90), 149 (89), 125 (100); ^{1}H-NMR (Table 1); ^{19}F-NMR δ = −93.17 ppm, app. t of d (J = 14.1, 2.4 Hz). The chemical composition of $C_{21}H_{28}F_2O_2$ was: calculated: C, 72.0%; H, 8.0%; F, 10.85%; found: C, 71.84%; H, 7.9%; F, 10.64%. The other isomers of methyl 4,4-difluororetinoate could not be crystallized. The 13-*cis* methyl 4,4-difluororetinoate showed UV max (hexane) 347 nm (ε 45,200) (Fig. 2); ^{19}F-NMR δ = −93.36 ppm, app. t of d (J = 14.1, 2.8 Hz).

TABLE 2. Spectroscopic properties of isomers of methyl 4,4-difluororetinoate separated by HPLC[a]

Fraction No.	Retention Time	Relative Percent	λ_{max} nm (Hexane)	Assignment
	min			
1	25.1	24	347	13-*cis*
2	28.6	9	343	
3	32.0	15	340	
4	34.1	1		
5	39.7	15	335	
6	43.6	36	340	all-*trans*
Methyl retinoate	24.6		350	all-*trans*

[a] Methyl 4,4-difluororetinoate purified by column chromatography on alumina was subjected to HPLC on a straight phase Partisil column developed with 1% ethyl acetate in hexane.

Preparation of 4,4-difluororetinol (6)

A stirred solution of methyl all-*trans* 4,4-difluororetinoate (50 mg, 0.4 mmol) in 5 ml of cold dry diethyl ether was treated with approximately 15 mg of $LiAlH_4$. The yellow solution immediately became very pale. After the addition of crushed ice, the ether layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated to give 4,4-difluororetinol as an oil (40 mg, 89% yield). The product was purified by HPLC on a μBondapak column, with a retention time of 1.6 min or 6.9 min, respectively, when methanol or methanol–water 4:1 was used as the eluant. The yield of 4,4-difluororetinol after HPLC was 35 mg. 4,4-Difluororetinol showed the expected UV max of 325 nm in hexane (ε 52,100) (Fig. 2) and the following mass spectral peaks: 322, M^+ (29), 304 (21), 291 (67), 145 (65), 119 (75), 107 (69), 95 (69), 81 (62), 69 (100). The identity of 4,4-difluororetinol was further confirmed by converting it into the acetate derivative (**7**) as described below.

Preparation of 4,4-difluororetinyl acetate (7)

Two procedures were used to prepare 4,4-difluororetinyl acetate. *Procedure I:* A solution containing 25 mg (0.07 mmol) of 4,4-difluororetinol in 0.6 ml of ice-cold pyridine was treated with 0.2 ml of freshly distilled acetic anhydride. After the solution was warmed to room temperature for 1 hr, crushed ice was added, and the product was extracted with hexane. The hexane extract was washed with water, dried over anhydrous Na_2SO_4, and roto-evaporated to give an oil, which was dissolved in 0.5 ml of acetone and purified by TLC using hexane–diethyl ether 4:1. 4,4-Difluororetinyl acetate (23 mg) (R_f = 0.72) showed a λ_{max} of 325 nm in hexane and a molecular ion at 364 in its mass spectrum. More detailed characterization is given under procedure II.

Procedure II: Five g (15.2 mmol) of all-*trans* retinyl acetate was oxidized with 25 g of precipitated MnO_2 (0.28 mol) for 20–30 hr (12). The resulting 4-oxoretinyl acetate (1.18 g of oil, 23% yield), eluted from a deactivated alumina column (100 g) with 20% ether in hexane, showed λ_{max} of 330 nm (broad) and 270 nm in hexane and of 348 nm (ε 49,500) and 280 nm (ε 22,500) in methanol. Prominent mass spectral peaks were: 342, M^+ (73), 327 (8), 282 (100), 267 (67), 145 (73), 94 (65).

4-Oxoretinyl acetate (1.026 g, 2.98 mmol) in the form of an oil was then treated with 0.485 g (3 mmol) of DAST at 50°C for 16–20 hr as just described. The difluorinated product, obtained in 5–10% yield, was eluted from an alumina column (50 g) with 5% ether in hexane. Unreacted 4-oxoretinyl acetate could be recovered with 15–20% ether in hexane. The product was resolved into three peaks with retention times of 15.5, 17.4, and 19.2 min by HPLC on a Partisil column using 5% ethyl acetate in hexane. The first and third peaks, tentatively identified as the 13-*cis* and all-*trans* isomers, respectively, accounted for 25% and 55%, respectively, of the recovered product. The second peak, which presumably contained other isomers, accounted for the remaining 20%. The range of overall recoveries of all-*trans* 4,4-difluororetinyl acetate from **9** in five experiments was 3–5%. The spectroscopic properties of all-*trans* 4,4-difluororetinyl acetate, obtained as a pale yellow oil, were: UV max (hexane) 325 nm (ε 54,400); (methanol) 325 nm; MS: m/z 364, M^+ (48), 344 (4), 304 (32), 289 (22), 145 (63), 132 (46), 94 (100); ^{19}F-NMR, δ = −93.23 ppm, app. t of d (J = 14.1, 2.8 Hz) (**Fig. 3**); ^{1}H-NMR (Table 1).

Preparation of 4,4-difluororetinyl palmitate (8)

A solution of 4,4-difluororetinol (10 mg, 0.03 mmol) in 2 ml of ice-cold pyridine was treated dropwise with 1 ml of a solution containing a drop (0.05 ml) of palmitoyl chloride dissolved in 20% toluene in pyridine. After the

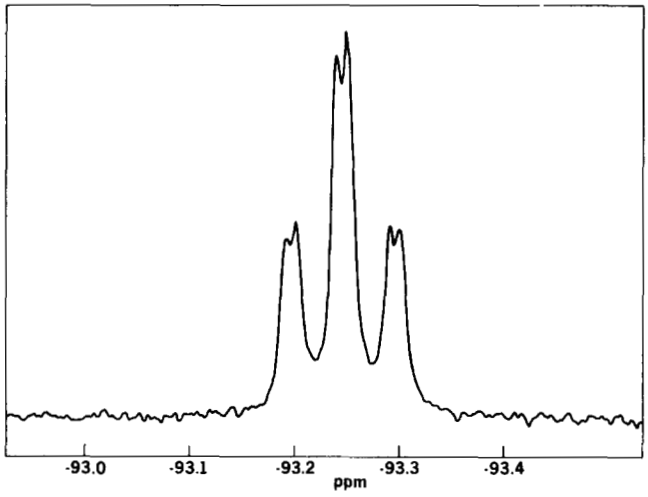

Fig. 3. ^{19}F-NMR spectrum of 4,4-difluororetinyl acetate in $CDCl_3$ (reference $CFCl_3$).

solution was warmed to room temperature for 30 min, crushed ice was added, and the product was extracted with diethyl ether. The ether extract was washed with water, dried over anhydrous Na_2SO_4, and roto-evaporated to yield an oil, which was dissolved in 0.5 ml of methanol. The palmitate ester (t_r = 4.97 min) was separated from unconverted difluororetinol (t_r = 1.6 min) by HPLC a on μBondapak column using methanol as the eluant. Difluororetinyl palmitate obtained as an oil (12 mg) showed UV max (hexane) 325 nm (ε 53,700); MS: m/z 560, M^+ (0.3), 540 (0.05), 304 (17), 289 (12), 256 (2), 145 (57), 132 (39), 119 (26), 94 (100).

4,4-Difluororetinoic acid (5)

A mixture of isomeric methyl esters of **4** (25 mg, 0.07 mmol) was dissolved in 1 ml of 1 M KOH in H_2O–MeOH 1:9, diluted with 1 ml of MeOH and warmed at 50°C for 2 hr. After acidification of the solution with 6 N HCl, retinoids were extracted with ether, dried over Na_2SO_4, and concentrated to an oil. The oil was dissolved in ether, diluted with hexane, added to a silica gel column (1.5 × 20 cm, wet-packed with hexane) and developed with increasing amounts of ether (5–60%) in hexane. In their order of elution, the compound, amount, and eluant were: **4** (1.3 mg, 5–10% ether); **3** (0.8 mg, 15–20% ether), **5** isomers (11.6 mg, 30–35% ether), and **11** (7.8 mg, 50–60% ether). 4-Oxoretinoic acid (**11**), in accord with earlier studies (17), showed UV max (methanol) at 356 nm and at 280 nm; MS, m/z 314, M^+ (100), 299 (29), 291 (49), 269 (13), 253 (12), 215 (12), 163 (75), 111 (49), 69 (41). 4,4-Difluororetinoic acid in CH_2Cl_2–hexane gave an orange-yellow solid: mp 155–165°C; UV max (MeOH) 350 nm (ε 47,000); MS: m/z 336, M^+ (10), 316 (47), 296 (15), 271 (8), 255 (19), 236 (33), 171 (52), 163 (98), 111 (88), 73 (100); ^{19}F-NMR, δ = −93.42 ppm, app. t of d (J = 12.2, 2.4 Hz). Based on the relative intensity of the ^{1}H-NMR signals of the C-14 proton at 5.82 ppm (all-*trans*) and at 5.70 ppm (13-*cis*), 4,4-difluororetinoic acid (**5**) was presumed to be a 3:2 mixture of all-*trans* and 13-*cis* isomers (Table 1). The retention times of 13-*cis* and all-*trans* isomers of **5** on a M9 column (20% ethyl acetate in hexane at 4 ml/min) during HPLC were 12.7 and 15.7 min, respectively.

Stability of difluoro analogs

Solutions containing 20 μg/ml of **4, 6,** or **7** in MeOH or MeOH–H_2O 4:1 were diluted with an equal volume of 0.02 M or 0.2 M KOH in MeOH and kept at 37°C for 1 hr. After being neutralized with HCl, aliquots were analyzed by reverse-phase HPLC for the formation of **5** and **11** from **4, 12** from **6,** and **6** and **12** from **7.** Similar studies were conducted in MeOH–H_2O 9:1 and in 0.01 and 0.1 M HCl in MeOH–H_2O 9:1.

In 10–100 mM alkali at 37°C, difluororetinol was converted at an appreciable rate (10–35%/hr) to 4-oxoretinol. Under the same conditions, the difluoroesters lost fluorine at a slower rate (10%/hr). At pH 7 in aqueous methanol at 37°C, the rate of fluorine loss from the difluororetinol and the esters was very slow (<1%/day). With 10–100 mM hydrochloric acid, difluororetinol forms compounds similar to anhydroretinol, whereas the difluoroesters only slowly (<10%/hr) gave 4-oxo derivatives.

DISCUSSION

In the past, analogs of vitamin A, or of an aromatic retinoid, have been prepared with fluorine atoms introduced in the C-10, C-12, and C-14 positions (8, 9). We have prepared two additional derivatives, retinoyl fluoride (18–20) and the series of 4,4-difluoro derivatives described in this report.

Because of the sensitivity of retinoids to oxidation, isomerization, and in the case of analogs of retinol to acid-catalyzed dehydration, the selection of suitable conditions for fluorination of specific positions in retinol was crucial. DAST, which efficiently replaces the hydroxyl groups of many alcohols and carboxylic acids with fluorine under relatively mild conditions (15, 16), was found, in our hands, to convert retinoic acid in good yield to retinoyl fluoride (18–20). In the preparation of DAST, particular care should be taken during its purification by distillation, inasmuch as the concentrated residue reacts violently with atmospheric moisture. The commercially available product must be handled prudently in a ventilated hood.

The introduction of fluorine into the C-4 position of retinol proved to be more difficult. Treatment of methyl 4-hydroxyretinoate with DAST in methylene chloride, for example, gave several nonfluorinated products. Similarly, when all-*trans* methyl 4-oxoretinoate was heated with DAST in an appropriate solvent as previously described (16), no fluorination occurred. When methyl 4-oxoretinoate was heated with DAST in the absence of a solvent, however, an isomeric mixture of methyl 4,4-difluororetinoate was formed in 5–10% yields. The mixture was resolved into six fractions by HPLC (Table 2). On the basis of ^{1}H-NMR spectra (Table 1), the all-*trans* configuration was assigned to the crystalline compound obtained from fraction 6 and the 13-*cis* configuration to the purified compound found in fraction 1. As shown in Table 1, the chemical shifts of protons in the conjugated chains of known all-*trans* methyl retinoate and of methyl 4,4-difluororetinoate from fraction 6 are essentially the same, whereas the C-12 proton in fraction 1 shows a downfield shift of 1.0–1.5 ppm, characteristic of 13-*cis* isomers (21, 22). The presence of fluorine in the 4,4-difluoro compounds was confirmed by ^{19}F-NMR spectra (Fig. 3).

Treatment of methyl 4,4-difluororetinoate with base gave mainly 4,4-difluororetinoic acid (**5**), but also some 4-oxoretinoic acid (**11**). Although obtained in a crystalline state, **5** contained about 60% of all-*trans* and 40% of 13-*cis* isomers on the basis of ^{1}H-NMR spectral signals. Reduction of all-*trans* methyl 4,4-difluororetinoate with lithium aluminum hydride gave 4,4-difluororetinol (**6**), which in turn was treated with acetic anhydride or palmitoyl chloride in pyridine to yield the acetate (**7**) or the palmitate (**8**) ester, respectively. Warming 4-oxoretinyl acetate (**9**) with DAST also gave difluororetinyl acetate (**7**) directly. The all-*trans* configuration was assigned to the major HPLC fraction of difluororetinyl acetate on the basis of the similarity of chemical shift values for protons in the conjugated chain of 4,4-difluororetinyl acetate and of known all-*trans* retinyl acetate (**10**) (Table 1). The ^{19}F-NMR spectra of 4,4-difluororetinyl acetate and its coupling constants were very similar to those shown by the other 4,4-difluoro derivatives that we prepared. The 4,4-difluoro derivatives of vitamin A were found to be more polar than the corresponding nonfluorinated derivatives, and could be readily separated from them by HPLC.

4,4-Difluororetinol and its acyl esters were quite stable under neutral conditions in aqueous methanol, e.g., >99% remained at 37°C after 1 day. In alkali, the expected defluorination to 4-oxoretinol occurred quickly, and in acid, 4,4-difluororetinol was readily dehydrated with the loss of fluorine to give a compound similar to anhydroretinol, with a λ_{max} at 370 nm. The difluoro-esters were somewhat more stable to acid or base than was the free alcohol. The relatively high stability of difluororetinol under neutral conditions encouraged us to study the biological activity and metabolism of this compound. In the rat growth bioassay, all-*trans* 4,4-difluororetinyl acetate showed significant biological activity (26%). Its metabolism in rats, including storage as an ester in the liver, was found to be similar to, but not identical with, that of all-*trans* retinyl acetate. These biological studies will be reported separately (23).

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REFERENCES

1. Moore, T. 1957. Vitamin A. Elsevier, Amsterdam.
2. Orfanos, C. E., O. Braun-Falco, E. M. Farber, C. Grupper, M. K. Polano, and R. Schuppli. 1981. Retinoids. Springer-Verlag, Berlin.
3. Bollag, W., and A. Matter. 1981. From vitamin A to retinoids in experimental and clinical oncology: achievements, failures, and outlook. *Ann. NY Acad. Sci.* **359:** 9–23.
4. Sporn, M. B., and D. L. Newton. 1979. Chemoprevention of cancer with retinoids. *Federation Proc.* **38:** 2528–2534.
5. Lotan, R. 1980. Effects of vitamin A and its analogs (retinoids) on normal and neoplastic cells. *Biochim. Biophys. Acta.* **605:** 33–91.
6. Hill, D. L., and C. J. Grubbs. 1982. Retinoids as chemopreventive and anticancer agents in intact animals (review). *Anticancer Res.* **2:** 111–124.
7. Sheppard, W. A., and C. M. Sharts. 1969. Organic fluorine chemistry. W. A. Benjamin, New York. 454–463.
8. Pawson, B. A., K. Chan, J. DeNoble, R. L. Han, V. Piermattie, A. C. Specian, and S. Srisethnil. 1979. Fluorinated retinoic acids and their analogs. 1. Synthesis and biological activity of (4-methoxy-2,3,6-trimethylphenyl) nonatetraenoic acid analogs. *J. Med. Chem.* **22:** 1059–1067.
9. Lovely, A. J., and B. A. Pawson. 1982. Fluorinated retinoic acids and their analogs. 3. Synthesis and biological activity of aromatic 6-fluoro analogs. *J. Med. Chem.* **25:** 71–75.
10. Liu, R. S. H., H. Matsumoto, A. E. Asato, M. Denny, Y. Shichida, T. Yoshizawa, and F. W. Dahlquist. 1981. Synthesis and properties of 12-fluororetinal and 12-fluororhodopsin. A model system for ^{19}F NMR studies of visual pigments. *J. Am. Chem. Soc.* **103:** 7195–7201.
11. Liu, R. S. H., and H. Matsumoto. 1982. Fluorine-labeled retinals and rhodopsins. *Methods Enzymol.* **81:** 694–698.
12. Roberts, A. B., and C. A. Frolik. 1979. Recent advances in the in vivo and in vitro metabolism of retinoic acid. *Federation Proc.* **38:** 2524–2527.
13. Frolik, C. A. 1981. In vitro and in vivo metabolism of all-*trans* and 13-*cis* retinoic acid in the hamster. *Ann NY Acad Sci.* **359:** 37–44.
14. Roberts, A. B. 1981. Microsomal oxidation of retinoic acid in hamster liver, intestine and testes. *Ann. NY Acad Sci.* **359:** 45–53.
15. Markovskij, L. N., V. E. Pashinik, and A. V. Kirsanov. 1973. Application of DAST in the synthesis of fluoroorganic compounds. *Synthesis.* 787–789.
16. Middleton, W. J. 1975. New fluorinating reagents. Dialkylaminosulfurfluorides. *J. Org. Chem.* **40:** 574–578.
17. Barua, A. B., and M. C. Ghosh. 1972. Preparation and properties of 4-oxoretinoic acid and its methyl ester. *Tetrahedron Lett.* **18:** 1823–1825.
18. Barua, A. B., and J. A. Olson. 1981. The synthesis and biological activity of fluorinated analogs of vitamin A. *Federation Proc.* **40:** 1803 (Abstract).
19. Barua, A. B., and J. A. Olson. 1982. The metabolism of 15-fluororetinone in rats. *Federation Proc.* **41:** 387 (Abstract).
20. Barua, A. B., and J. A. Olson. 1983. Preparation, characterization, biological activity and metabolism of all-*trans* retinoyl fluoride. *Biochim. Biophys. Acta.* **757:** 288–295.
21. Patel, D. J. 1969. 220 MHz proton nuclear magnetic resonance spectra of retinals. *Nature.* **221:** 825–828.
22. Vetter, W., G. Englert, N. Rigassi, and U. Schwieter. 1971. IV. Spectroscopic methods. *In* Carotenoids. O. Isler, editor. Birkhauser Verlag, Basel. 189–266.
23. Barua, A. B., and J. A. Olson. 1984. Metabolism and biological activity of all-*trans*-4,4-difluororetinyl acetate. *Biochim. Biophys. Acta.* In press.