Preparation of retinamides by use of retinoyl fluoride

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Abstract Retinoyl fluoride (*2*) prepared from retinoic acid (*1*) by reaction with diethylaminosulfurtrifluoride is a stable crystalline compound not easily hydrolyzed by water. By reacting retinoyl fluoride with water-soluble amines in the presence of sodium bicarbonate, retinamide (*4*), N-retinoyl glycine (*6*), N-retinoyl DL-phenylalanine (*7*), α-N-retinoyl-L-lysine (*11*), N-retinoyl 4-aminophenol (4-hydroxyphenylretinamide) (*8*), and N-retinoyl-2-amino-2-deoxy-D-glucose (2-deoxy-D-glucose-2-retinamide) (*9*) have been prepared in good yields and characterized by UV absorption, ^{1}H NMR, IR spectra, mass spectrometry, and elemental analysis. — **Barua, A. B., and J. A. Olson.** Preparation of retinamides by use of retinoyl fluoride. *J. Lipid Res.* 1985. **26:** 258–262.

Supplementary key words vitamin A • retinoyl chloride

Vitamin A plays an important role in growth, vision, and reproduction. Its various natural and synthetic analogs, the retinoids, also have the ability to inhibit tumor cell growth and enhance the differentiation of certain malignant cells (1). Among various retinoids, the retinamides have received considerable attention for their activity in the prevention of chemically-induced cancer of the skin, mammary gland (2), and urinary bladder (3) in experimental animals. Although amides may be synthesized by several procedures (4), the acylation of amines with acid chlorides is the most common route. By treatment of an appropriate amine with retinoyl chloride under anhydrous conditions, several all-*trans* and 13-*cis* retinamides have been prepared (5–7) and characterized spectroscopically (8). Because retinoyl fluoride, unlike retinoyl chloride, is not readily hydrolyzed by water (9), it can be used in the formation of N-retinoyl derivatives of water-soluble amines of biological interest. In this study, we report the preparation in good yield of several retinamides, some of which are novel, by treating the appropriate amine with retinoyl fluoride (9) in the presence of aqueous sodium bicarbonate.

MATERIALS AND METHODS

Chemicals and solvents

Compounds used and their sources are as follows: all-*trans* retinoic acid, DL-phenylalanine, adenine hydrochloride, D (+) glucosamine hydrochloride, and type L-α-phosphatidylethanolamine from *E. coli*, ~98% (Sigma Chemical Co., St. Louis, MO); diethylaminosulfurtrifluoride (DAST) and 4-aminophenol (Aldrich Chemical Co., Milwaukee, WI); silica gel for dry column chromatography, activity III/30 mm (Woelm Pharma, Eschwege, West Germany, supplied by Universal Scientific Inc., Atlanta, GA); ammonium hydroxide (28–30%) and methanol (Fischer Scientific Co., Fair Lawn, NJ); L-lysine monohydrochloride (Nutritional Biochemical Corp., Cleveland, OH). All other chemicals, solvents, and reagents were of analytical or reagent grade.

Chromatographic techniques

For thin-layer chromatography (TLC), compounds spotted on Brinkmann silica gel G plates (0.25 mm, 20 × 20 cm) were developed with mixtures of hexane and acetone (4:1 or 2:1). For column chromatography (CC), silica gel for dry column chromatography was wet-packed with hexane and developed with hexane, with a mixture of hexane and diethyl ether or with other organic solvents as defined in the Experimental Section. High pressure liquid chromatography (HPLC) was performed with an instrument consisting of a Waters Associates U6-K injector and a 6000A pump, a Perkin-Elmer LC 75 variable wavelength detector, and a Hewlett Packard 3390A integrator. A Whatman ODS-2 column (0.4 × 30 cm) was

Abbreviations: TLC, thin-layer chromatography; CC, column chromatography; HPLC, high pressure liquid chromatography; DAST, diethylaminosulfurtrifluoride.

used for reverse phase separations, with methanol or methanol–water (4:1 or 7:3) containing 10 mM ammonium acetate as the eluant at a flow rate of 2 ml/min.

Physicochemical and spectrometric analysis

Ultraviolet spectra were recorded with a Shimadzu model UV-240 spectrophotometer. Infrared (IR) spectra were obtained by using an IBM FT-IR spectrophotometer as KBr pellets. ^{1}H NMR spectra were run in a Bruker WM-300 or Nicolet NT-300 MHz instrument in $CDCl_3$ (if not otherwise stated) with tetramethyl silane (TMS) as an internal standard. ^{31}P NMR spectra were recorded with a Bruker WM-300 MHz instrument. Mass spectra (MS) of compounds were obtained by using a Finnegan model 4000 GC/MS instrument. The size of each mass peak relative to the base peak of 100 is given in parentheses following the mass ion value. Melting points (uncorrected) were determined in a Fischer-Johns melting point apparatus.

All the retinamides described in this paper have the all-*trans* configuration, if not otherwise stated.

EXPERIMENTAL SECTION

Preparation of retinoyl fluoride (*2*) Fig. 1

To a cold (−70°C) solution of retinoic acid (*1*) (2 g, 6.6 mmol) in diethyl ether (120 ml), an ice-cold solution of DAST (1.1 g, 6.8 mmol) was added dropwise. After the solution was warmed to room temperature, the solvent was removed by roto-evaporation. Argon was passed thru the oil, in a hood, to get rid of side products that react exothermically with silica gel. The red oil was dissolved in a small volume of hexane and poured onto a column of silica gel (2 × 4 cm), from which retinoyl fluoride was quickly eluted with 5% diethyl ether in hexane. After solvent removal, the resultant oil (95%, 1.91 g) was dissolved in a small volume of hexane, from which all-*trans* retinoyl fluoride (*2*) crystallized at −20°C. Yield: 1.5 g (75%); mp 68–71°C; UV_{max} (hexane) 382 nm (ε 49,800). The other physical properties of retinoyl fluoride have been previously described (9).

Preparation of retinamide (*4*)

To a solution of retinoyl fluoride (*2*) (400 mg, 1.32 mmol) in diethyl ether (2 ml), methanol (1 ml) and aqueous ammonia (0.5 ml, 28–30%) were added. Although a yellow solid separated immediately, the mixture was stirred for 1 hr at room temperature to maximize the yield. The solid was filtered off and dissolved in diethyl ether. The ether phase was washed with water, dried over Na_2SO_4 and evaporated to dryness. The resultant solid was dissolved in the minimum volume of methanol and poured onto a silica gel column. Small amounts of methyl

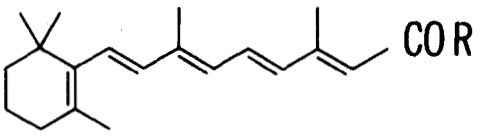
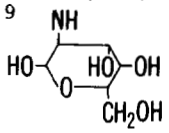
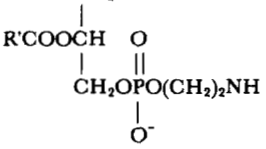


Fig. 1 Structure of retinamides.

No.	R	Name
1	OH	Retinoic acid
2	F	Retinoyl fluoride
3	OCH_3	Methyl retinoate
4	NH_2	Retinamide
5	NHOH	N-Hydroxyretinamide
6	$NH(CH_2)CO_2H$	N-Retinoyl glycine
7	$NHCH(CO_2H)CH_2C_6H_5$	N-Retinoyl phenylalanine
8	$NH(C_6H_4)OH$ (1,4)	N-(4-Hydroxyphenyl)retinamide
9		2-Deoxy-D-glucose-2-retinamide
10		N-Retinoyl phosphatidyl-ethanolamine
11	$NHCH(CO_2H)(CH_2)_4NH_2$	N-Retinoyl-α-lysine
12	$NH(CH_2)_4CH(CO_2H)NH_2$	N-Retinoyl-ε-lysine

retinoate (*3*) and retinoic acid (*1*) were first eluted with 5% and 30% diethyl ether in hexane, respectively, whereas retinamide (*4*) was eluted with 30% acetone in hexane. After removal of the solvent, retinamide was crystallized from ethanol. Yield: 326 mg (82%); mp 174–175°C. UV_{max} (methanol) 347 nm (ε 42,100); ^{1}H NMR δ 6.95 (dd, 1, J = 15, 11Hz, H-11); 6.26 (d, 1, J = 16Hz, H-7), 6.25 (d, 1, J = 11Hz, H-12), 6.24 (d, 1, J = 15Hz, H-10), 6.12 (d, 1, J = 16Hz, H-8), 5.72 (s, 1, H-14), 5.40 (s, 2, $CONH_2$), 2.36 (d, 3, J = 0.78Hz, C-13 CH_3), 2.02 (m, 2, C-4 CH_2), 1.99 (s, 3, C-9 CH_3), 1.71 (s, 3, C-5 CH_3), 1.61 (m, 2, C-3 CH_2), 1.48 (m, 2, C-2 CH_2), 1.02 (s, 6, C-1 $(CH_3)_2$). IR: 3383, 3179, 2924, 1645, 1609, 1582, 1408, 1371, 1329, 962 cm^{-1}. MS (m/z) 299 (M^+) (84), 284, 255 (15), 201 (38), 162 (50), 110 (100). Analysis calculated for $C_{20}H_{29}ON$: C, 80.26; H, 9.69; N, 4.68. Found: C, 80.02; H, 9.80; N, 4.60.

General reaction procedure for the formation of N-retinoyl amines

Retinoyl fluoride (75–400 mg) was dissolved in 2–5 ml of diethyl ether and diluted to 10 ml with methanol. A slight molar excess of the selected amine (50–363 mg) or its hydrochloride was dissolved or suspended in a saturated aqueous solution of $NaHCO_3$, such that the final solution was slightly alkaline. The solutions of the amine and retinoyl fluoride were mixed and stirred under

alkaline conditions at room temperature until retinoyl fluoride was no longer detected by TLC (1–6 hr). Amide formation was usually indicated by a change in the color of the solution from orange-yellow to pale lemon-yellow. The solution was then acidified with 1 N HCl and extracted with diethyl ether or butanol. The organic extract, after washing with 1 N HCl and then with water, was dried with anhydrous Na_2SO_4.

Preparation of N-retinoyl glycine (*6*)

The washed, dried diethyl ether extract of the reaction between glycine (150 mg, 2.0 mmol) and retinoyl fluoride (400 mg, 1.3 mmol) was evaporated to give a mixture of *1,3* and N-retinoyl glycine (*6*). CC gave pure *6* as a low melting yellow solid (260 mg, 54%). N-Retinoyl glycine was further purified by HPLC (methanol–water 7:3), t_R = 20 min (broad). UV_{max} (methanol) 345 nm (ϵ 49,100): ^{1}H NMR δ 6.95 (dd, 1, J = 15, 11Hz, H-11), 6.38 (ill-defined t, 1, J = ~6Hz, CONH), 6.26 (d, 1, J = 16Hz, H-7), 6.24 (d, 1, J = 15 Hz, H-12), 6.17 (d, 1, J = 11Hz, H-10), 6.12 (d, 1, J = 16Hz, H-8), 5.75 (s, 1, H-14), 4.1 (d, 2, J = 4Hz, $NHC\underline{H}_2$), 2.34 (s, 3, C-13 CH_3), 2.02 (m, 2, C-4 CH_2), 1.98 (s, 3, C-9 CH_3), 1.71 (s, 3, C-5 CH_3), 1.6 (m, 2, C-3 CH_2), 1.46 (m, 2, C-2 CH_2), 1.02 (s, 6, C-1 $(CH_3)_2$). IR: 3305, 2928, 2862, 1738, 1643, 1607, 1574, 1533, 1360, 1196, 966 cm^{-1}. MS (m/z) 357 (M^+) (100), 339, 267, 255 (20), 159 (44), 121 (45), 76 (54).

The methyl ester of N-retinoyl glycine prepared by treating N-retinoyl glycine with diazomethane was purified by CC and crystallized from hexane: mp 78–80°C, UV_{max} (methanol) 347 nm (ϵ 46,300). Analysis calculated for $C_{23}H_{33}O_3N$: C, 74.39; H, 8.89; N, 3.77. Found: C, 74.16; H, 9.02; N, 3.54.

Preparation of N-retinoyl DL-phenylalanine (*7*)

The washed, dried diethyl ether extract of the reaction between retinoyl fluoride (302 mg, 1 mmol) and DL-phenylalanine (245 mg, 1.5 mmol) was evaporated to give a fine yellow powder that was dissolved in benzene, diluted with hexane, and subjected to CC. N-Retinoyl phenylalanine (*7*) was eluted with 50–60% diethyl ether in hexane. Evaporation of the solvent gave N-retinoyl DL-phenylalanine as a light yellow powder. Yield: 165 mg (37%); mp 132–135°C. UV_{max} (methanol) 347 nm (ϵ 41,700); ^{1}H NMR δ 7.16–7.32 (m, C_6H_5 and $CHCl_3$), 6.93 (dd, 1, J = 15, 11Hz, H-11), 6.21 (d, 1, J = 16Hz, H-7), 6.15 (d, 1, J = 15Hz, H-12), 6.12 (d, 1, J = 16Hz, H-8), 6.10 (d, 1, J = 11Hz, H-10), 5.93 (d, 1, J = 7Hz, NH), 5.63 (s, 1, H-14), 4.90 (q, 1 J = 6Hz, NCH), 3.19 and 3.23 (pair of q, 2, J = 6Hz, $C_6H_5C\underline{H}_2$), 2.32 (s, 3, C-13 CH_3), 2.02 (m, 2, C-4 CH_2), 1.99 (s, 3, C-9 CH_3), 1.71 (s, 3, C-5 CH_3), 1.62 (m, 2, C-3 CH_2), 1.46 (m, 2, C-2 CH_2), 1.02 (s, 6, C-1 $(CH_3)_2$). IR: 3500–3200 (broad), 3020, 2950, 2875, 1730, 1640, 1525, 1450, 1390, 1370, 1260, 1190, 1130, 970, 880, 840, 740, 710 cm^{-1}. MS (m/z) 447 (M^+) (31), 429 (2), 324 (0.3), 298 (2), 282 (7), 267 (14), 258 (29), 165 (100). Analysis calculated for $C_{29}H_{37}NO_3$: C, 77.85; H, 8.27; N, 3.13. Found: C, 77.58; H, 8.04; N, 2.95.

Preparation of N-retinoyl L-lysine (α, *11*)

The washed, dried butanol extract of the reaction between L-lysine HCl (363 mg, 2 mmol) and retinoyl fluoride (400 mg, 1.32 mmol) was evaporated to give a yellow solid containing *1*, *3*, *11*, and presumably *12*. The solid was dissolved with difficulty in methanol–CH_2Cl_2–diethyl ether (2:1:1) and poured onto a column of silica gel. After elution of *1* and *3*, the N-retinoyl L-lysines (*11* and presumably *12*) in 27% yield were eluted with methanol–CH_2Cl_2 (1:1 and 2:1, respectively).

α-N-Retinoyl L-lysine (*11*) was obtained as a yellow solid (92 mg, 13%); mp 212–214°C (decom.). UV_{max} (methanol) 345 nm (ϵ 36,400); ^{1}H NMR δ 6.94 (dd, 1, J = 15, 11Hz, H-11), 6.24 (d, 1, J = 16Hz, H-7), 6.22 (d, 1, J = 15Hz, H-8), 5.75 (ill-defined t, 1, J = ~6Hz, CONH), 5.67 (s, 1, H-14), 5.01 (q, 1, J = 6Hz, $C\underline{H}$ (COOH)), 4.65 (pair of q, 2, J = ~6Hz, $C\underline{H}_2$ $(CH_2)_3$), 3.31 (q, 4, J = 6Hz, $(C\underline{H}_2)_2CH_2$), 2.35 (s, 3, C-13 CH_3), 2.01 (m, 2, C-4 CH_2), 1.98 (s, 3, C-9 CH_3), 1.71 (s, 5, C-5 CH_3 and $C\underline{H}_2NH_2$ or $CH_2N\underline{H}_2$), 1.59 (m, 2, C-3 CH_2), 1.44 (m, 2, C-2 CH_2), 1.02 (s, 6, C-1 $(CH_3)_2$), 1.01 (s, 2, $C\underline{H}_2NH_2$ or $CH_2N\underline{H}_2$). IR: 3500, 3373, 2949 (broad), 1601, 1590, 1516, 1410, 1346, 1013, 557 cm^{-1}.

The methyl ester of *11* was obtained by treatment of *11* in diethyl ether (sparingly soluble) with diazomethane. The ^{1}H NMR spectrum of the methyl ester was the same as *11*, except that an additional signal at 3.74 ppm (s, OCH_3) was present. The other lysine derivative, presumably ϵ-N-retinoyl lysine (*12*), has not been well characterized.

Preparation of N-retinoyl 4-aminophenol (N-4-hydroxyphenyl)retinamide) (*8*)

The washed, dried diethyl ether extract of the reaction between 4-aminophenol (218 mg, 2 mmol) and retinoyl fluoride (302 mg, 1 mmol) was evaporated to give a red oil. The oil was dissolved in a small volume of diethyl ether and subjected to column chromatography. After methyl retinoate and retinoic acid were removed with 5–50% diethyl ether in hexane, the main yellow band containing N-(4-hydroxyphenyl)retinamide (*8*) was eluted with 70–80% diethyl ether in hexane. After solvent removal, crude *8* as an oil was dissolved in 1 ml of diethyl ether and precipitated by the addition of hexane. N-(4-Hydroxyphenyl)retinamide was filtered and crystallized, solvated with a molecule of ethanol, from ethanol and hexane. Yield: 367 mg (83%); mp 175–177°C. UV_{max} (methanol) 362 (ϵ 57,100), 231 (ϵ 14,000) nm; ^{1}H NMR

δ 7.25–7.35 (m, C_6H_4 and $CHCl_3$), 7.14 (s, 1, NH or OH), 6.97 (dd, 1, J = 15, 11Hz, H-11); 6.27 (d, 2, J = 16Hz, H-7 and H-8), 6.14 (d, 1, J = 15Hz, H-12), 6.13 (d, 1, J = 11Hz, H-10), 5.63 (s, 1, OH or NH), 5.78 (s, 1, H-14), 3.44 (q, 2, J = 7Hz, $CH_3C\underline{H}_2OH$), 2.41 (s, 3, C-13 CH_3), 2.02 (m, 2, C-4 CH_2), 2.00 (s, 3, C-9 CH_3), 1.71 (s, 3, C-5 CH_3), 1.63 (m, 2, C-3 CH_2), 1.48 (m, 2, C-2 CH_2), 1.24 (t, 3, J = 7Hz, $C\underline{H}_3CH_2OH$), 1.03 (s, 6, C-1 $(CH_3)_2$). IR: 3400, 3300, 3050, 2950, 2910, 2850, 2780, 1650, 1620, 1590, 1525, 1520, 1440, 1360, 1310, 1240, 1180, 970, 835, 520 cm^{-1}. MS (m/z) 391 (M^+) (4), 376, 297, 283 (2), 292 (2), 268 (3), 255 (10), 202 (26), 161 (29), 135 (55), 119 (41), 109 (100). Analysis calculated for $C_{26}H_{33}NO_2$, C_2H_5OH: C, 76.88; H, 8.92; N, 3.20. Found: C, 77.33; H, 8.90; N, 3.27.

Preparation of N-retinoyl-2-amino-2-deoxy-D-glucose (2-deoxyglucose-2-retinamide) (*9*)

The washed, dried butanol extract of the reaction between D-glucosamine · HCl (283 mg, 1.32 mmol) and retinoyl fluoride (400 mg, 1.32 mmol) was evaporated to give a yellow oil. Crude 2-deoxyglucose-2-retinamide in methanol was chromatographed on a silica gel column from which *9* was eluted with 20% methanol in diethyl ether. The yield of the yellow oil was 520 mg (79%). The oil solidified after being kept under hexane at −20°C for several days; mp of the yellow solid 165–168°C. UV_{max} (methanol) 348 nm (ε 39,600); ^1H NMR δ 7.14, 5.53, and 5.23 (broad s, 3, glucosamine moiety), 6.84 (dd, 1, H-11), 6.05–6.32 (m, 4, H-7, 8, 10, 12), 5.79 (s, 1, H-14), 3.43–4.3 (overlapping s and m, complex, 9, glucosamine moiety), 2.28 (s, 3, C-13 CH_3), 1.99 (m, 2, C-4 CH_2), 1.93 (s, 3, C-9 CH_3), 1.68 (s, 3, C-5 CH_3), 1.58 (m, 2, C-3 CH_2), 1.4 (m, 2, C-2 CH_2), 1.00 (s, 6, C-1 $(CH_3)_2$). IR: 3319 (strong and broad), 2926, 1632, 1609, 1578, 1529, 1442, 1359, 1065, 1026, 966 cm^{-1}. MS (m/z) 443 (M^+-H_2O) (0.3), 425 (0.3), 341 (10), 255 (10), 56 (100). Analysis calculated for $C_{26}H_{39}O_6N$: C, 67.67; H, 8.45; N, 3.03. Found: C, 67.37; H, 8.03; N, 2.67.

Other N-retinoyl amines

Retinoyl fluoride also reacted easily with hydroxylamine, adenine, and L-α-phosphatidylethanolamine under similar conditions to yield products tentatively identified as N-retinoylhydroxylamine (N-hydroxyretinamide) (*5*), N-retinoyl-6-adenine, and N-retinoyl phosphatidylethanolamine (*10*).

With hydroxylamine hydrochloride, retinoyl fluoride gave two compounds: 1) a yellow solid, obtained as a minor product, that absorbed maximally at 358 nm in methanol, mp 108–110°C, MS (m/z) 315, 300, 283, 269; and 2) a brownish oil, obtained as the major product, which was tentatively assigned an N-hydroxyretinamide (*5*) structure. UV_{max} (methanol) 348 nm; MS (m/z) 315 (M^+), 297 (M^+-H_2O), 282, 269, 255.

N-Retinoyl adenine, presumably the 6-amino derivative, was obtained as a red solid in a fair yield (26%); mp 190°C (d), UV_{max} (methanol) 397 nm, 255 nm. The compound was fairly soluble in methanol, sparingly soluble in CH_2Cl_2 and diethyl ether, and insoluble in hexane. The ^1H NMR spectrum (0–10 ppm) and C,H analysis did not allow an unambiguous interpretation of structure. Another adenine derivative, yellow in color and eluted before the red product on silica gel columns, was also formed. Yield: 7%, mp 175–178°C, UV_{max} (methanol) 397 nm and 255 nm. Again, the ^1H NMR and C,H analysis did not provide a characterization of structure.

N-Retinoyl phosphatidylethanolamine (*10*) was obtained as a yellow oil in about 20% yield, UV_{max} 347 nm, ^{31}P NMR δ 1.44 (t, J = ~7Hz) in $CDCl_3$ with H_3PO_4 as the reference compound. The compound was soluble in diethyl ether and in methanol–chloroform 1:1, but not in hexane.

DISCUSSION

In the search for retinoids with good therapeutic and chemopreventive activity combined with low toxicity, retinamides, i.e, N-retinoyl derivatives of various amines, have shown considerable promise (10). In synthesizing and evaluating the chemical and biological properties of retinoyl fluoride (9), we found that retinoyl fluoride reacts with all amines we have tested to produce retinamides in varying yields. Thus, by the reaction of retinoyl fluoride with ammonia, glycine, lysine, phenylalanine, glucosamine, and 4-aminophenol, we have been able to prepare retinamide, N-retinoyl glycine, α-N-retinoyl-lysine, N-retinoyl phenylalanine, 2-deoxyglucose-2-retinamide, and 4-hydroxyphenylretinamide, respectively. These retinamides have been characterized by analysis of their UV-visible, IR, ^1H NMR, and mass spectra and elemental analyses.

In their ultraviolet spectra, the retinamides show absorption maximum at approximately 345 nm in methanol, except for N-(4-hydroxyphenyl)retinamide, which absorbed at 362 nm, and the N-retinoyl adenine derivatives, which absorbed at approximately 400 nm. In their infrared spectra, the retinamides showed asymmetric and symmetric N–H stretching vibrations at 3300–3500 cm^{-1}. In the case of the amino acid derivatives, these N–H stretching vibrations were overlapped by OH stretching vibrations in very broad bands. The C=O stretching vibrations of the acyl amides (amide I band) and the N–H bending vibrations of the amide II band absorbed intensely in the 1500–1750 cm^{-1} region.

Conclusive evidence about the structures of most retinamides was derived from ^1H NMR spectra. In the case of the glucosamine derivative *9*, however, no assignments were made because the signals appeared as complex multiplets and overlapping singlets. Moreover, minor weak signals were observed due possibly to the presence of more

than one anomeric form. Similarly, the ^{1}H NMR spectra of the adenine derivatives were not readily interpreted.

The yields of the various retinamides vary between 27–83%, depending on the nature of the starting amine. With diethyl ether-soluble or methanol-soluble amines, the yields are high, often above 80%. In this regard, retinoyl fluoride compares favorably with retinoyl chloride for the preparation of retinamides. The use of retinoyl fluoride offers other advantages as well, namely: 1) retinoyl fluoride is a stable, easily handled solid, whereas retinoyl chloride is an unstable liquid (11); 2) retinoyl fluoride can be used in a medium containing water, whereas retinoyl chloride requires anhydrous conditions; and 3) with retinoyl fluoride free amino acids are directly used as reactants, whereas with retinoyl chloride the ethyl esters of amino acids are used to enhance their solubility, e.g., in the synthesis of N-retinoyl glycine (7). Thereafter, the ethyl ester must be hydrolyzed.

During the formation of retinamides, some retinoic acid is invariably formed along with very small amounts of methyl retinoate. These impurities can easily be eliminated by column chromatography. The pH of the reaction medium is critical for amide formation. The presence of too much aqueous sodium bicarbonate, for example, enhances the hydrolysis of retinoyl fluoride at the expense of amide bond formation. Consequently the yield of highly water-soluble amines tends to be lower ($\sim$30%) than that of nonpolar amines. On the other hand, if the medium is not basic, no retinamide is formed. With the liberation of hydrofluoric acid during amide bond formation and the coincident generation of some retinoic acid, the medium becomes acidic. Thus the pH of the medium must be monitored and more bicarbonate added as necessary.

The reaction is usually completed in 1 hr, but may take several hours in some instances. The progress of the reaction can be visually monitored by a color change from dark orange color to pale lemon-yellow, or better by testing for the disappearance of retinoyl fluoride from the reaction mixture by TLC.

Retinoyl fluoride also reacts with hydroxylamine, adenine, phosphatidylethanolamine, and other amines to yield N-retinoyl derivatives. The formation of these latter compounds, although not as yet adequately characterized, further demonstrates the generality of the reaction. Under some conditions, however, the reaction may be quite specific. For example, retinoyl fluoride reacts with opsin to yield a N-retinoyl-opsin that does not form rhodopsin with 11-*cis* retinal (12). The ϵ-amino group of the lysine residue that binds 11-*cis* retinal reacts with all-*trans* or 9-*cis* retinoyl fluoride in a highly preferential manner (13). Similarly, retinoyl fluoride reacts preferentially with some proteins of liver cytosol (D. Cooper, A. B. Barua, and J. A. Olson, unpublished observations). Thus retinoyl fluoride may prove to be a useful reagent not only in the chemical synthesis of N-retinoyl amines but also in a biological context.

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