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Synthesis and Properties of Some 13-*cis*- and All-*trans*-retinamides

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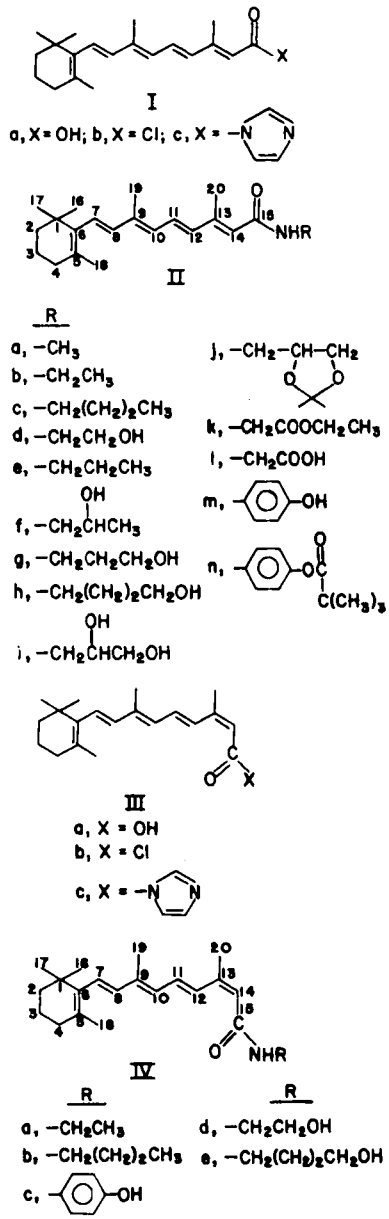
Abstract □ Several 13-*cis*-retinamides were synthesized from 13-*cis*-retinoic acid *via* either 13-*cis*-retinoyl chloride or 13-*cis*-1-retinoylimidazole. All-*trans*-retinoylglycine was prepared from all-*trans*-retinoyl chloride and ethyl glycinate. Detailed procedures were developed for the preparation of other all-*trans*-retinamides on a large scale for studies of the chemoprevention of cancer.

Keyphrases □ 13-*cis*-Retinamides—synthesis and chemical properties □ All-*trans*-retinamides—synthesis and chemical properties □ Synthesis—13-*cis*- and all-*trans*-retinamides, chemical properties

Compounds of the vitamin A group (retinoids) are essential for normal cellular differentiation and for the growth of epithelial tissues (1–4). During recent years, many studies, reviewed by Sporn and co-workers (3–9) and Bollag and co-workers (10–12), have shown that retinoid deficiency in animals enhances susceptibility to chemical carcinogenesis, that epithelia in certain organ cultures develop preneoplastic lesions in the absence of retinoids, and that administration of certain retinoids may prevent or reduce carcinogen-induced neoplasia in epithelial tissues of animals, as well as preneoplasia (6, 13–16) in organs in culture. In particular, certain derivatives and analogues of all-*trans*-retinoic acid (tretinoin, also known as vitamin A acid; Ia) exert a prophylactic (and, in some cases, a therapeutic) effect on the development of preneoplastic and malignant epithelial lesions (*e.g.*, 11, 16–25). Among the more interesting derivatives of retinoic acid and its analogues are the amides (retinamides and retinamide analogues). The purpose of this report is to describe the preparation of several all-*trans*-retinamides (II) and 13-*cis*-retinamides (IV) for long-term studies of chemoprevention in animals. Only one 13-*cis*-retinamide, the primary amide (IV, R = H), had been previously described (without a synthesis procedure) in the literature (26).

RESULTS AND DISCUSSION

The preparation of all-*trans*-retinamides IIa–d (27) and IIe (28) has been briefly outlined in the patent literature, but the characterization data (mp, UV) were sparse. The 4-hydroxyphenyl amide (all-*trans*-*N*-(4-hydroxyphenyl)retinamide; IIm) was synthesized originally by Gander and co-workers (24, 28). The preparation of IIf–l has not been described previously, but IIf–i



and II l were among the retinoids evaluated in organ culture by Newton *et al.* (16) and were synthesized, originally, elsewhere[1].

All-*trans*-retinamides have been prepared (24–31) from retinoic acid (Ia) *via* all-*trans*-retinoyl chloride (Ib) in benzene (29), benzene–dimethylformamide (24, 28), or ether (27). The acid chloride (Ib) was prepared initially from Ia and phosphorus trichloride in benzene (29) and subsequently from Ia and thionyl chloride in pyridine–ether (30). Most of the retinamides described here were obtained by modifying the phosphorus trichloride method and adapting it to large-scale syntheses. The volume of benzene may be reduced considerably below that used in the original procedure (29) for the preparation of a retinamide. Although IIa–i and IIk have the same general structure, the isolation procedures used to obtain good yields of the various retinamides differ in detail. Compound IIh could also be obtained *via* Ib, but it was more conveniently prepared from 1-(all-*trans*-retinoyl)imidazole (Ic), which was prepared from Ia and 1,1′-carbonyldiimidazole (32). The dihydroxypropyl amide (IIi) was prepared by both methods; the isopropylidene derivative (IIj) was prepared from IIi by a procedure adapted from nucleoside chemistry (33). All-*trans*-*N*-retinoylglycine (II l) was prepared by treating IIb with an excess of ethyl glycinate free base and saponifying the resulting ester (IIk).

Differences among some of the all-*trans*-retinamides in their sensitivity to atmospheric oxygen were observed. Since all have the same basic retinoid structure, these differences are probably due principally to differences in crystal structure and particle size (*Experimental*). The 2,3-dihydroxypropyl amide (IIi) appeared to be the most sensitive of the all-*trans*-retinamides to deliberate exposure to air, whereas the ethyl amide (IIb) and 4-hydroxyphenyl amide (IIm) appeared to be among the least susceptible to atmospheric oxygen.

The 13-*cis*-retinamides (IV) were prepared from 13-*cis*-retinoic acid (IIIa) by way of either 13-*cis*-retinoyl chloride (IIIb) or 1-(13-*cis*-retinoyl)imidazole (IIIc). In initial runs, it was shown by NMR and HPLC that 13-*cis*-retinoic acid (IIIa) could be converted to 13-*cis*-retinoyl chloride (IIIb) and thence to 13-*cis*-*N*-ethylretinamide (IVa) without causing isomerization of the 13-*cis* structure to the all-*trans* isomer. Compounds IVa–c could be prepared *via* IIIb in yields of 74–82% from IIIa. 1-(13-*cis*-Retinoyl)imidazole (IIIc) was prepared from IIIa and 1,1′-carbonyldiimidazole by a procedure similar to that employed for the preparation of the all-*trans* isomer. A specimen of IIIc was purified, but less pure specimens, which might have contained varying amounts of imidazole, were satisfactory for the formation of 13-*cis*-retinamides. The conversion *via* IIIc was quite satisfactory, and superior to the acid chloride method, for the preparation of the hydroxyalkyl amides (IVd,e). In later, large-scale runs, IIIc was prepared and used *in situ;* consequently, the use of IIIc for the preparation of 13-*cis*-retinamides is comparable in simplicity to the acid chloride method. Yields of IVd,e were 60–70%.

Although amides are simple derivatives and IVa–e are all representatives of the same general structure, the preparation of these 13-*cis*-retinamides was not without difficulties. The most serious problem was the occasional formation during the preparation or isolation of IVa and IVb of considerable amounts (10–25%) of the all-*trans* isomer. The reasons for these occasional lapses were not apparent, but it is evident that the synthesis of a 13-*cis*-retinamide should be carefully monitored by HPLC and NMR to ensure the absence of significant amounts of the corresponding all-*trans* isomers. Other operational difficulties were caused by idiosyncracies of individual structures. The 4-hydroxybutyl derivative (IVe) could be crystallized initially only from cyclohexane; it then retained cyclohexane (~1:1) tenaciously (*Experimental*). Once a crystalline specimen was obtained free of cyclohexane, IVe could be readily recrystallized from acetonitrile–water. The 2-hydroxyethyl amide (IVd), which is isolated as a syrup, dissolves in hexane at room temperature and crystallizes. The crystalline solid is then only sparingly soluble in boiling hexane, but it is soluble in cyclohexane and can be recrystallized from the latter solvent. Compound IVc showed a tendency to retain benzene, the reaction solvent. The butyl amide (IVb) resisted crystallization and appeared to undergo considerable degradation during attempts to purify and crystallize it until seed crystals were finally obtained.

Other than differences owing to absorptions of the R groups, there is little, or no, variation in the NMR spectra[2] of the all-*trans*-retinamides (II) or of the 13-*cis*-retinamides (IV). The major differences between the 100-MHz ^{1}H-NMR spectra of the all-*trans* (IIa–l) and the 13-*cis* amides (IVa, b, d, and e) are the pronounced downfield shift of the C-12 proton signal and the upfield shifts of the C-14 and C-20 proton signals in the spectra of the 13-*cis* amides. For example, the C-12 proton signal in the spectrum of all-*trans*-*N*-ethylretinamide (IIb) is part of a multiplet (CH of C-7, C-8, C-10, and C-12 + NH) at δ 6.0–6.4 ppm, and the C-20 and C-14 proton signals are approximate singlets at δ 2.37 and δ 5.71 ppm, respectively; whereas the corresponding proton signals in the spectrum of the 13-*cis* isomer (IVa) are δ 7.84 (d, C-12), 2.01 (s, C-20), and 5.57 ppm (s, C-14). The ^{13}C-NMR spectra of the all-*trans*-retinamides are in excellent agreement with one another and with data from spectra of other all-*trans*-retinoids determined by Englert (35) and assigned with the use of lanthanide shift reagents. The greatest differences in the ^{13}C-NMR spectra (obtained in $CDCl_3$) of the 13-*cis*-retinamides (IVa, b, d, and e) relative to the spectra of the all-*trans* amides (IIa–l) are the downfield shift of C-20 (~7.2 ppm) and the upfield shift of C-12 (5–6 ppm). Significant upfield shifts were also observed in the C-13 (1.8–2.1 ppm), C-14 (1.7–1.8 ppm), and C-15 (0.4–0.5 ppm) peaks in the 13-*cis*-retinamide spectra. These differences are exemplified by chemical shifts in the ^{13}C-NMR spectra of the all-*trans*- and 13-*cis*-*N*-ethyl amides: C-20, δ 13.54 (IIb) and 20.73 ppm (IVa); C-12, δ 135.79 (IIb) and 129.7–130.6 ppm [one of four closely spaced peaks (C-5, C-10, C-11, and C-12) in the spectrum of IVa]; C-13, δ 147.89 (IIb) and 145.98 ppm (IVa); C-14, δ 121.95 (IIb) and 120.23 ppm (IVa); C-15, δ 167.12 (IIb) and 166.66 ppm (IVa).

The UV spectra[2] of the aliphatic all-*trans*-retinamides have an absorption maximum at 347–348 nm with a molar absorptivity (ϵ) of ~50,000–51,000. This maximum is shifted slightly (2–4 nm) to longer wavelengths in the spectra of the 13-*cis*-retinamides, and the molar absorptivity is lower (range: 42,200–47,700). Both the absorption maxima and the absorptivity are greater in the *N*-aryl amides. The IR spectra[2] of all of these retinamides (II and IV)

[1] Compounds IIf–i were first supplied for biological evaluation (16) by BASF Aktiengesellschaft, Ludwigshafen am Rhein, West Germany, and compounds IIm,n, by Johnson and Johnson, New Brunswick, N.J.

[2] Detailed spectrometric data for the individual retinamides (IIa–n and IVa–e) are listed in a complementary publication (34).

show at least one strong band in the region of 965–985 cm^{-1}. This band is due to the C—H out-of-plane vibrations of the *trans*-disubstituted ethylene groups (36). The molecular ion (M) is one of the most intense peaks observed above *m/z* 200 in the mass spectra of the retinamides (II and IV) when the spectra[2] are measured in the electron-impact mode at a direct-probe temperature of ~20°C. Additional characteristic, though less intense, peaks observed are M − 15, loss of a methyl group; M − 123, loss of the 2,6,6-trimethyl-1-cyclohexenyl group; and M − R, *m/z* 298, loss of the R group, when R is not an aryl group. In all of the spectra in the region above *m/z* 200, other characteristic peaks that are usually more intense than the peak at M − 15 or at *m/z* 298 are *m/z* 282 (M − NHR − H), 267 (282 − CH_3), 255 (M − CONHR), 241 (M − CH_3 − CONHR + H), 240, 239, 225 (240 − CH_3), 213, 211, and 201. Other prominent peaks, as well as less intense peaks, also appear in the region above *m/z* 200, and numerous strong peaks are present in all of the spectra in the region below *m/z* 200.

Most of these retinamides have been evaluated in the hamster trachael organ assay and are highly active (16). Several have been shown to have chemopreventive activity (24, 25) *in vivo* and to be significantly less toxic than is all-*trans*-retinoic acid (37). All-*trans*-retinoylglycine, (II l) like all-*trans*- and 13-*cis*-retinoic acids, is a strong inducer of retinoic acid metabolism (38) and is more active against hyperplasia in mouse prostate organ cultures than is all-*trans*-retinoic acid (39).

EXPERIMENTAL

All operations involved in the preparation, isolation, purification, and transfer of retinoids were performed in an atmosphere, or under a current, of dry nitrogen or argon. All such operations were also performed in dim light or photographic darkroom light and, insofar as possible, with containers wrapped with aluminum foil or with black cloths. All retinoids were stored in an atmosphere of argon or nitrogen in hermetically sealed containers at −20°C or −80°C.

Melting temperatures were determined in capillary tubes[3]. UV spectra[4] were determined with ethanol solutions (except for IIm, where methanol was employed). IR spectra[5,6] were determined from specimens in pressed potassium bromide disks. Mass spectral data were taken from low-resolution, electron-impact spectra determined at 70 eV[7]; the direct-probe temperature was 20°C. ^{1}H-NMR spectra were determined at 100 MHz[8]. ^{13}C-NMR spectra were determined at 25.2 MHz[8] or 100.6 MHz[9]. Both the ^{1}H- and ^{13}C-NMR spectra were recorded from $CDCl_3$ solutions except for the spectra of IIm and IVc, which were recorded from Me_2SO-d_6 solutions. The more salient spectroscopic data are summarized in the discussion.

TLC was performed on plates of fluorescing silica gel[10], and developed plates were examined with UV lamps (254 and 365 nm) unless otherwise indicated in parentheses. Other pertinent information (amount applied, developing solvent, other methods of detection) is given parenthetically at the appropriate places in the experimental procedures.

HPLC was performed with a components system[11] and an integrator[12] or with a complete system[13] and was monitored by UV at 340 nm, unless otherwise indicated. Reverse-phase HPLC was performed on columns of octadecylsilane (ODS) chemically bonded to silica: column A, μBondapak[11] C_{18}, 10-μm particle size; column B, Spherisorb[14] ODS, 5-μm particle size; column C, Partisil[15] ODS-2, 10-μm particle size. HPLC eluting solvents were: solvent A, gradient 60:40 acetonitrile–water (solution *a*) → 98:2 acetonitrile–water (solution *b*), 0% solution *b* → 100% solution *b*; solvent B, 85:15 acetonitrile–1% aqueous ammonium acetate, isocratic; solvent C, 80:20 acetonitrile–1% aqueous ammonium acetate, isocratic.

All-*trans*-retinoyl Chloride (Ib)—All-*trans* retinoic acid (Ia, 400 g, 1.33 mol) was suspended in 4 L of dry, reagent-grade benzene in a dry 12-L, 3-necked, round-bottom flask fitted with a motor-driven stirrer, thermometer, addition funnel, and inlet and exit tubes for nitrogen. A solution of 129.3 g (0.94 mol) of phosphorus trichloride in 1.6 L of dry benzene was added in a fast, continuous stream to the vigorously stirred mixture at ~25°C. No increase in temperature was observed during the addition; stirring was continued under a brisk current of nitrogen and with occasional warming to maintain the temperature at 25–30°C. The retinoic acid slowly dissolved, and the mixture changed from a suspension to an orange solution plus a gummy precipitate (phosphorus compounds) that generally collected on the walls of the flask. On this scale, all of the retinoic acid dissolved within ~3 h. The solution was stirred for an additional 1 h, stirring was discontinued, and the gummy precipitate was allowed to settle.

While the retinoyl chloride solution was being prepared, a dry benzene or benzene–dimethylformamide solution of the amine required for the desired retinamide was prepared under nitrogen and cooled. The retinoyl chloride solution was decanted under nitrogen into an addition funnel for introduction into the appropriate amine solution.

All-*trans*-*N*-methylretinamide (IIa)—By the procedure described for the preparation of IIb (below), methylretinamide IIa was prepared from Ib and methylamine, recrystallized from methanol, and dried *in vacuo* at room temperature to yield 11.3 g from 15 g of Ia (72%), mp 175–176°C. Since TLC revealed a trace impurity and NMR showed the presence of a small amount of methanol, the product was recrystallized from ethanol (300 mL) to yield 8.6 g (55%), mp 175–176°C [lit. (27) mp 174–175°C]; TLC: 1 spot (99:1 chloroform–methanol); HPLC: 100% (column A, solvent A, monitored at 345 and 254 nm).

Anal.—Calc. for $C_{21}H_{31}NO$: C, 80.47; H, 9.97; N, 4.47. Found: C, 80.42; H, 10.03; N, 4.40.

All-*trans*-*N*-ethylretinamide (IIb)—The general procedure (25) for the preparation of IIb and similar all-*trans*-retinamides is illustrated by the following procedure. A solution of all-*trans*-retinoyl chloride (Ib) prepared, as described above, from 400 g (1.33 mol) of Ia was added during 0.5 h to a stirred solution of 595 g (13.2 mol) of ethylamine in 1100 mL of dry, reagent-grade benzene at 10°C. The temperature was maintained at 10–15°C with an ice–salt bath, and the reaction mixture was stirred an additional 0.5 h at 10°C. Cold water (4 L) was added during ~10 min, stirring was continued for 15 min, and the mixture was concentrated *in vacuo* with a large rotating evaporator to remove the benzene and excess ethylamine. The yellow, crystalline solid suspended in the aqueous mixture was collected by filtration, pressed by suction with a rubber sheet to remove a yellow, oily material, washed thoroughly on the filter with water, pressed again with a rubber sheet to remove as much water as possible, and dried further *in vacuo*. The solid was dissolved in hot methanol (~5 L), the solution was filtered, and the hot filtrate was diluted with warm water to the cloud point. The mixture was allowed to cool to room temperature and was then stored in a refrigerator overnight. The yellow, crystalline product was collected by filtration, washed with cold methanol–water (4:1), and dried *in vacuo* to constant weight to yield 356 g (82%), mp 138–140°C [lit. (27) mp 137–138°]; TLC: 1 spot (99:1 chloroform–methanol); HPLC: 99.7–100% (column B with solvent B or column A or C with solvent C, monitored at 350 and 254 nm).

All-*trans*-*N*-butylretinamide (IIc)—Compound IIc was prepared by the procedure described for IIb from butylamine (580 mL in 1 L of dry benzene) and a solution of Ib (prepared from 400 g of Ia, 81.3 mL of phosphorus trichloride, and 5 L of dry benzene). The mixture was stirred for 45 min and then washed thoroughly with cold water (1.5 L). The organic layer was washed again with cold water (1 L), concentrated to ~1 L, filtered to collect a yellow precipitate, and concentrated further to obtain a second crop[16]. The crude product (450 g) obtained by combining the two crops was recrystallized from methanol–water (9:1, 2.7 L). The yellow, crystalline solid was collected by filtration, washed with cold methanol–water, and dried *in vacuo* at room temperature to yield 376 g (79%) of IIc, mp 92–94°C [lit. (27) mp 92–93°C]; TLC: 1 spot (99:1 chloroform–methanol); HPLC: >99.9% (column B, solvent B).

Anal.—Calc. for $C_{24}H_{37}NO$: C, 81.07; H, 10.49; N, 3.94. Found: C, 81.03; H, 10.68; N, 3.75.

All-*trans*-*N*-(2-hydroxyethyl)retinamide (IId)—Compound IId was prepared by the procedure described for IIb from ethanolamine (dried over molecular sieves, 600 mL in 1 L of dry benzene) and a solution of Ib (prepared from 350 g of Ia in 4.8 L of dry benzene). After a reaction period of 45 min at 10°C, the product (pasty solid) was isolated (difficult filtrations) as was IIb, washed with cold methanol–water (1:1), triturated with cold methanol–water (2:1, 400 mL), and recrystallized (*cf.*, IIb) twice from methanol–water (3:1). The yellow crystals were dried *in vacuo* at room temperature to yield 335 g (84%) of IId, mp 139–141°C [lit. (27) mp 138–139°C]; TLC: 1 spot with tailing (95:5 chloroform–methanol); HPLC: 99.8–100% (column B and solvent B or column C and solvent D).

Anal.—Calc. for $C_{22}H_{33}NO_2$: C, 76.93; H, 9.68; N, 4.08. Found: C, 76.63; H, 9.88; N, 3.98.

[3] Mel-Temp apparatus.
[4] Cary Model 17 UV spectrophotometer.
[5] Perkin-Elmer Model 621 spectrometer.
[6] Nicolet Model MX-1 Fourier Transform spectrometer.
[7] Varian/MAT Model 113 A mass spectrometer.
[8] Varian XL-100-15 NMR spectrometer.
[9] Bruker WH-400 NMR spectrometer.
[10] Silica Gel GF precoated TLC plates (fluorescent), 250 μm thickness; Analtech Inc., Newark, Del.
[11] Waters Associates, Milford, Mass.
[12] Model 3380 S; Hewlett-Packard.
[13] Model 1084 B; Hewlett-Packard.
[14] Spectra-Physics, Houston, Tex. or Universal Scientific Inc., Atlanta, Ga.
[15] Whatman Inc., Clifton, N.J.

[16] Compound IIc could also be isolated by evaporating benzene and butylamine from the water-organic mixture (*cf.*, IIb) after addition of the second portion of water and then including the yellow, oily layer to crystallize.

All-*trans*-*N*-propylretinamide (IIe)—Compound IIe was prepared from Ia (50 g) and *n*-propylamine (59 g) by the procedure described for IIb. The crude product (51 g) was recrystallized from ethanol-water (5:1, 600 mL) to yield 45.8 g (81%), mp 113–114°C [lit. (28) mp 113–115°C]; HPLC: 99.4% (column B, solvent B).

Anal.—Calc. for $C_{23}H_{35}NO$: C, 80.88, H, 10.33; N, 4.10. Found: C, 81.18; H, 10.69; N, 3.89.

(±)-All-*trans*-*N*-(2-hydroxypropyl)retinamide (IIf)—Compound IIf was prepared by the procedure described for IIb from (±)-1-amino-2-propanol (420 mL in 1.8 L of dry benzene) and a solution of Ib (prepared from 300 g of Ia, 60.8 mL of phosphorus trichloride, and 3.8 L of dry benzene). After a reaction time of 1 h at 10–15°C and concentration of the reaction mixture to ~50% of the original volume, the addition of cold water (1.5 L) to the stirring mixture caused precipitation of the product. The mixture was allowed to stand in an ice bath, and the precipitate was then collected by filtration, pressed with a rubber sheet by suction on the filter, washed with water, and dried. The benzene layer was separated from the filtrate, concentrated, and diluted with cold water (500 mL). A second portion of IIf was separated by filtration as above. The two portions were combined (279 g) and recrystallized from 2:1 acetonitrile-water (3 L). The pale yellow, crystalline product was collected by filtration and dried at room temperature to yield 237 g (67%) of IIf, mp 116–117°C; TLC: 1 spot (95:5 chloroform-methanol); HPLC: 99.4–99.9% (column B, solvent B).

Anal.—Calc. for $C_{23}H_{35}NO_2$: C, 77.27; H, 9.87; N, 3.92. Found: C, 77.58; H, 10.11; N, 4.08.

All-*trans*-*N*-(3-hydroxypropyl)retinamide (IIg)—A mixture of Ia (200 g, 0.67 mol), 1,1′-carbonyldiimidazole (124 g, 0.79 mol), and anhydrous benzene (2 L) was stirred at room temperature for 2 h. The resulting solution was warmed at 50°C in a water bath for 15 min, cooled to room temperature, and slowly added to a cold (10°C), stirred solution of 3-amino-1-propanol (260 mL, 3.6 mol) in anhydrous benzene (1 L). The reaction mixture was stirred at 10°C for 2 h, allowed to stand at room temperature overnight, and diluted with ether (~1 L) to minimize emulsification. The resulting solution was washed with water (2 × 1 L), and the organic layer was dried over sodium sulfate and concentrated to a low volume. The yellow, finely divided precipitate was collected by filtration, washed with small amounts of benzene and ether, and dried *in vacuo* to yield 195 g. A second crop (18 g) was obtained from the filtrate (total yield of crude IIg = 89.4%). The two portions were combined and dissolved in hot ethyl acetate (1 L). The pale yellow precipitate was filtered from the cool mixture and dried *in vacuo* to yield 154 g (65%) of IIg, mp 110–111°C (cap., inserted at 95°C); TLC: 1 spot (95:5 chloroform-methanol); HPLC: 99.7–100% (column B, solvent B).

Anal.—Calc. for $C_{23}H_{35}NO_2$: C, 77.27; H, 9.87; N, 3.92. Found: C, 77.30; H, 10.05; N, 4.14.

All-*trans*-*N*-(4-hydroxybutyl)retinamide (IIh)—The procedure was similar to that described for the preparation of IIg except that the ratio of the amine (4-aminobutanol, 0.97 mol) to Ia (0.33 mol, 100 g) was lower. After the benzene-ether solution had been washed with cold water (3×), it was concentrated to a viscous oil that crystallized after seed crystals were added. The yellow, crystalline solid was triturated with cyclohexane-hexane (1:2, 100 mL), collected by filtration, dried *in vacuo* (weight, 96 g), and recrystallized from acetonitrile (hot). The pale yellow crystals were filtered from the cold mixture and dried *in vacuo* at room temperature to yield 93 g (73%) of IIh, mp 111–113°C (cap., inserted at 80°C); TLC: 1 spot (95:5 chloroform-methanol); HPLC: 99.8% (column B, solvent B).

Anal.—Calc. for $C_{24}H_{37}NO_2$: C, 77.58; H, 10.04; N, 3.77. Found: C, 77.76; H, 10.30; N, 3.65.

A polymorphic form of IIh was also obtained (from cyclohexane or acetonitrile), mp 95–96°C. The solid-state IR spectrum differed from, but was similar to, that of the higher-melting form. The two forms were shown to be identical by TLC, HPLC, ^{1}H-NMR, and ^{13}C-NMR.

(±)-All-*trans*-*N*-(2,3-dihydroxypropyl)retinamide (IIi)—A solution of all-*trans*-retinoyl chloride (prepared from 100 g of Ia, 21 mL of phosphorus trichloride, and 1300 mL of dry benzene) was added to a solution of 200 g of (±)-3-amino-1,2-propanediol in 1200 mL of dry benzene and 300 mL of dimethylformamide at a rate that did not cause the mixture temperature to exceed 20°C. The orange, two-phase mixture was stirred overnight at room temperature. The supernatent solution was decanted from the gummy amine hydrochloride and divided into approximately equal amounts in two 5-L separatory funnels, each containing 1 L of water and 1.5 L of ethyl acetate. The emulsions separated during standing, and the aqueous layers were removed. The organic layers were extracted repeatedly (5–8 times) with 600-mL portions of water. Additional amounts of ethyl acetate and small portions of sodium chloride were added, as needed, during these extractions to cause separation of the emulsions. The two organic layers were combined, and the solution was chilled in an ice bath, dried over anhydrous magnesium sulfate, and concentrated *in vacuo* to ~1 L. (Much magnesium sulfate was required to dry the solution and considerable heat was evolved.) Finely divided, yellow crystals formed, and 1 L of cyclohexane was added. After the mixture had been stored in a freezer overnight, IIi was collected by filtration, washed with 1:1 ethyl acetate-cyclohexane, and dried *in vacuo* at room temperature to constant weight to yield 103 g (83%), mp 101–104°C. Polymorphic forms of IIi were obtained: mp 83–85°C from acetonitrile; mp 101–104°C from ethyl acetate. The specimen described above (103 g) was recrystallized from acetonitrile to yield 98 g (79%) of IIi, mp 83–85°C; HPLC: 98–100% (column B, solvent B).

Anal.—Calc. for $C_{23}H_{35}NO_3$: C, 73.96; H, 9.44; N, 3.75. Found: C, 73.21; H, 9.64; N, 3.63.

Preparation of Derivative IIj—A solution prepared by carefully adding 16.74 mL of 60% perchloric acid to a stirred solution of 11.16 mL of 2,2-dimethoxypropane and 415 mL of anhydrous acetone was stirred for 5 min, (±)-all-*trans*-*N*-(2,3-dihydroxypropyl)retinamide (12.5 g) was added in one portion, and the resulting solution was stirred for 50 min. Pyridine (16.75 mL) was added, and the solution was concentrated *in vacuo* to a gummy residue that was partitioned between chloroform (500 mL) and saturated aqueous sodium bicarbonate (500 mL). The aqueous layer was reextracted twice with 250-mL portions of chloroform, and the chloroform solution (3 portions combined) was dried over magnesium sulfate and concentrated *in vacuo* to an orange syrup. A solution of the residual syrup in petroleum ether (125 mL, bp 30–60°C) was filtered and then refrigerated. A yellow precipitate was collected by filtration, washed with cold petroleum ether, and dried *in vacuo* at room temperature to give 11.8 g. Since TLC showed that this material still contained some of the starting material, it was chromatographed on a column of silica gel. The column was eluted with 95:5 chloroform-methanol, and the fractions containing IIj were located by TLC. Concentration of these fractions *in vacuo* left a yellow syrup (8.2 g) that was crystallized from petroleum ether (150 mL). Yellow crystals were filtered from the cold mixture, washed with petroleum ether, and dried *in vacuo* at room temperature to yield 6.02 g (43.5%) of IIj, mp 106–110°C; HPLC: 97.2% (column B, solvent B); UV_{max} (ethanol): 348 nm (ϵ 47,500).

All-*trans*-*N*-retinoylglycine Ethyl Ester (IIk)—A solution of 196 g (1.40 mol) of glycine ethyl ester hydrochloride in 200 mL of water was covered with 400 mL of ether. The mixture was cooled to 0°C in an ice bath, and 160 mL of 33% aqueous NaOH was added at such a rate that the temperature of the reaction mixture did not exceed 4°C. Immediately after the sodium hydroxide had been added, anhydrous potassium carbonate was added until the water layer was a pasty mass. The ether layer was decanted, and the residual mass was extracted twice with 250-mL portions of ether. The combined, cold, ether extracts were dried over a 9:1 mixture of anhydrous potassium carbonate and barium hydroxide and filtered through a pad of anhydrous magnesium sulfate. The total volume of the combined filtrate and ether washings was 900 mL. The solution was cooled to 5°C and used in the coupling reaction with Ib. (In an earlier, small-scale reaction, concentration of the dried, ether extracts afforded a fluid, colorless oil; examination of this product by MS and by ^{1}H-NMR indicated that little or no diketopiperazine was present.)

A solution of Ib in 450 mL of dry benzene (prepared from 45 g of Ia) was added to the vigorously stirred ethyl glycinate solution (described above) at a rate that did not cause the temperature to exceed 10°C. The mixture was then stirred at 10°C for 1 h. Ice-cold water (450 mL) was added in a dropwise manner, the two-phase mixture was concentrated *in vacuo* at ~35°C to remove the ether and benzene, and the fluid yellow syrup that remained in suspension was extracted into a minimum amount of ether. The ether layer was stirred vigorously with water (400 mL) to ensure thorough mixing, the mixture was concentrated *in vacuo* to remove the ether, and the water was decanted from the viscous syrup. This process was repeated, but the water-syrup mixture was refrigerated overnight before the water was decanted. An ether solution of the residual syrup was dried ($MgSO_4$) and concentrated *in vacuo* at 35°C to constant weight to yield 55 g (95%) of a yellow glassy material. Crystalline IIk was obtained by dissolving the glassy material in warm ethanol and storing the filtered solution at −15°C; mp 91–93°C; TLC: 1 spot (10:1 chloroform-acetone); HPLC: 99% (column A, solvent A).

Anal.—Calc. for $C_{24}H_{35}NO_3$: C, 74.76; H, 9.15; N, 3.63. Found: C, 74.39; H, 8.95; N, 3.36.

All-*trans*-*N*-retinoylglycine (II l)—A stirred solution of 15.0 g (38.9 mmol) of crude *N*-retinoylglycine ethyl ester in 265 mL of ethanol that contained 15.0 g of 85% KOH was heated at 60°C for 1 h. The reaction mixture was poured into 600 mL of water at 0°C, and the solution was extracted with ether (3 × 250 mL) to remove neutral and basic impurities. When the aqueous layer was acidified to pH 3 with 1.5 M sulfuric acid, a fluid oil separated. The mixture was extracted with 250 mL of ether, and the extract was washed twice with water and dried over anhydrous magnesium sulfate. Concentration of the dried extract afforded 13.5 g (97%) of a viscous orange syrup. Crystallization of the crude product twice from acetonitrile (about 10 ml/g) gave yellow microcrystals, mp 94–97°C; TLC: 1 spot (8:2:1 butanol-acetic acid-

water); HPLC: >98% (column A, solvent 1:1 acetonitrile–1% ammonium acetate).

Anal.—Calc. for $C_{22}H_{31}NO_3$: C, 73.91; H, 8.74; N, 3.92. Found: C, 73.68; H, 8.21; N, 3.78.

All-*trans*-*N*-(4-hydroxyphenyl)retinamide (IIm)—The preparation of IIm was based on the procedure of Gander and co-workers (24, 28) and is similar to the preparation of other all-*trans*-retinamides from IIb. The literature procedure (24, 28) was modified to adapt it to large-scale synthesis as follows. The precipitate of 4-aminophenol hydrochloride was filtered from the reaction mixture; the filtrate was washed with water, concentrated, and chilled; and the crude, crystalline product was collected (1–3 crops). These modifications obviate the need for the large quantities of ether used in the original procedure and permit the preparation of 300–400 g of IIm per run. (Some ether was added during the workup of some of the preparations of IIm in order to minimize emulsification.) The crude product was recrystallized from methanol–water and then from ethanol–water, mp 173–175°C; TLC: 1 spot (95:5 chloroform–methanol); HPLC: 99.9% (column B, solvent B).

Anal.—Calc. for $C_{26}H_{33}NO_2$: C, 79.77; H, 8.50; N, 3.58. Found: C, 79.55; H, 8.75; N, 3.54.

This compound evidently crystallizes in several polymorphic or solvated forms. Among many specimens prepared by the procedure outlined above, the mp of 173–175°C was typical, and the IR spectra of most of these specimens were identical; however, the solid-state IR spectra of a few specimens that melted over a 1–2°C range between 171°C and 176°C differed in fine structure from the typical spectrum. Therefore, there may be more than one form with a melting point in this range. Also, a form obtained by slow crystallization of IIm from methanol melted at 178–181°C and retained methanol. The form reported by Gander and co-workers (24) melted at 162–163°C and apparently is more soluble in chloroform than the typical form described above. All of these forms, including a specimen with mp 162–163°C (24), were shown by UV, ^{1}H-NMR, ^{13}C-NMR, MS, TLC, and HPLC to be identical with one another and to be IIm of high purity.

All-*trans*-*N*-[4-(pivaloyloxy)phenyl]retinamide (IIn)—This compound was prepared by acylating IIm with pivaloyl chloride in benzene–pyridine, pouring the reaction mixture into water, and recrystallizing the yellow precipitate from methanol–water and then from ethanol–water, mp 158–160°C; TLC: 1 spot (99:1 chloroform–methanol); HPLC: 99.9–100% (column B, solvent B).

Anal.—Calc. for $C_{31}H_{41}NO_3$: C, 78.28; H, 8.69; N, 2.94. Found: C, 78.23; H, 8.72; N, 2.71.

1-(13-*cis*-Retinoyl)imidazole (IIIc)—A mixture of 13-*cis*-retinoic acid (20 g, 66 mmol) and 1,1′-carbonyldiimidazole (12.4 g, 76 mmol) in benzene (300 mL) was stirred at room temperature for 3 h; gas evolution had ended by this time. The solution was warmed to 50°C for 15 min and then concentrated *in vacuo* to an orange syrup. A mixture of the residue and cyclohexane (250 mL) was stirred at room temperature, filtered to remove a beige solid, and concentrated to dryness under reduced pressure. A solution of the residue in cyclohexane (150 mL) was diluted with hexane (50 mL) and cooled with stirring in an acetone–dry ice bath at −10°C. A yellow precipitate was collected by filtration and dried to yield 18 g (77% yield of crude product). Recrystallization of a 13-g portion of this material from cyclohexane and refrigeration of the mixture afforded 7 g of a yellow solid, mp 70–85°C; HPLC: 98%. A second crop of 2.4 g (mp 75–80°C; HPLC: 96.6%) was obtained after the filtrate was diluted with hexane and refrigerated. A cyclohexane (150 mL) solution of 6.9 g of this material (crop 2 plus part of crop 1) was washed with water (2 × 50 mL), dried ($MgSO_4$), concentrated to ~30 mL, and stored at −15°C. A yellow crystalline solid was collected by filtration and dried *in vacuo* to yield 5.0 g of IIIc, mp 85–86°C (cap., inserted at 65°C); HPLC (column B, solvent B): 98.2% IIIc (retention time, 20.2 min) and 1.2% of an impurity (retention time, 17.6 min). A spike at δ 1.42 ppm on the upfield side of a multiplet in the ^{1}H-NMR spectrum indicated the presence of a small amount of cyclohexane in this specimen. A portion was crushed to a powder and dried thoroughly *in vacuo* prior to microanalysis.

Anal.—Calc. for $C_{23}H_{30}N_2O$: C, 78.82; H, 8.63; N, 7.99. Found: C, 78.94; H, 8.79; N, 8.26.

From another run beginning with 40 g of 13-*cis*-retinoic acid, compound IIIc was obtained in three crops of crystals from cyclohexane–hexane: 2.4 g, mp 77–80°C; 22.6 g, mp 73–83°C; and 13.3 g, mp 82–84°C. The total yield of IIIc satisfactory for use as an intermediate was 82%.

13-*cis*-*N*-Ethylretinamide (IVa)—13-*cis*-Retinoic acid (IIIa, 75 g, 0.25 mol, dried *in vacuo* over P_2O_5) was suspended in 700 mL of dry, reagent-grade benzene, and a solution of 15.2 mL of phosphorus trichloride in 200 mL of dry benzene was added rapidly to the vigorously stirred mixture. The reaction mixture was then stirred at 25°C for 3 h. During this time, 13-*cis*-retinoic acid slowly dissolved and a gummy precipitate formed. Stirring was discontinued, the precipitate was allowed to settle, and the supernatant solution was decanted into an addition funnel.

The acid chloride (IIIb) solution was added slowly to a stirring solution of 130 mL of ethylamine in 300 mL of dry benzene at 10°C, and stirring was continued for 0.5 h. Cold water (250 mL) was added with stirring, the layers were separated, and the aqueous layer was discarded. The benzene layer was washed with two more 250-mL portions of water and concentrated *in vacuo* to a viscous, yellow syrup. The syrup was dissolved in methanol (175 mL), and the solution was diluted slowly with water just short of the cloud point and then seeded with previously obtained crystals of IVa. After some crystallization had occurred, more water was added slowly with stirring (50–75 mL), and the mixture was refrigerated overnight. The precipitate (68 g) was collected by filtration, dried, and recrystallized by dissolving it in refluxing acetonitrile and adding water (~6:1 acetonitrile–water) to yield 64 g (78%), mp 114–115°C (cap., inserted at 95°C); TLC: 1 spot (99:1 chloroform–methanol); HPLC (column B, solvent B): >99.9%.

Anal.—Calc. for $C_{22}H_{33}NO$: C, 80.68; H, 10.16; N, 4.28. Found: C, 80.75; H, 10.44; N, 4.41.

13-*cis*-*N*-Butylretinamide (IVb)—A solution of 13-*cis*-retinoyl chloride (IIIb) [prepared as described above (IVa) from 40 g (133 mmol) of 13-*cis*-retinoic acid in 400 mL of dry benzene and 8.1 mL (93 mmol) of phosphorus trichloride in 155 mL of dry benzene] was added slowly to a vigorously stirred, cold (10°C) solution of 64 mL (650 mmol) of *n*-butylamine in 300 mL of dry benzene. Stirring was continued for 1 h, the mixture was extracted with cold water (2 × 500 mL), and the benzene layer was dried with anhydrous sodium sulfate and concentrated *in vacuo* to give a viscous, orange syrup. A solution of the syrup in 95% acetonitrile at room temperature was seeded with previously obtained crystals of IVb and refrigerated. The pale yellow, crystalline solid was collected by filtration, washed sparingly with cold 95% acetonitrile, and dried *in vacuo* to yield 35 g (74%), mp 77–78°C (cap., inserted at 50°C); TLC: 1 spot (98:2 chloroform–methanol); HPLC (column B, solvent B): >99.9%.

Anal.—Calc. for $C_{24}H_{37}NO$: C, 81.07; H, 10.49; N, 3.94. Found: C, 81.11; H, 10.69; N, 4.03.

13-*cis*-*N*-(4-Hydroxyphenyl)retinamide (IVc)—A solution of 13-*cis*-retinoyl chloride in benzene was prepared by the procedure described above for IVa from 65 g of 13-*cis*-retinoic acid (dried *in vacuo* overnight over P_2O_5), 13.2 mL of phosphorus trichloride, and 750 mL of dry benzene. The benzene solution of IIIa was added slowly to a cold (10°C), stirring solution of 100 g of 4-aminophenol in 400 mL of dimethylformamide (spectral grade), and the reaction mixture was allowed to warm to room temperature and stirred overnight. The mixture, containing precipitated 4-aminophenol hydrochloride, was stirred in an ice bath for 1 h before it was filtered to remove the precipitate. Water (400 mL) was added to the stirring filtrate, and the aqueous layer was separated and discarded. (Much of the product sometimes precipitated during the water extraction or during a second water washing.) The benzene layer was concentrated under reduced pressure to ~300 mL, cooled in an ice bath, and filtered to remove the yellow precipitate, which was washed with benzene and dried. This material (66 g) was combined with a second crop (14 g), obtained similarly from the filtrate and washings, and the total product was recrystallized from methanol–water. A refluxing ethanol solution of the recrystallized material (72 g) was diluted with water well past the point at which crystallization began, and the mixture was cooled to room temperature and then refrigerated. The yellow, crystalline precipitate was collected and dried to yield 70 g (82%) of IVc; mp 186–188°C (cap., inserted at 165°C); TLC: 1 spot (95:5 chloroform–methanol); HPLC (column B, solvent B): >99.7%.

Anal.—Calc. for $C_{26}H_{33}NO_2$: C, 79.77; H, 8.50; N, 3.58. Found: C, 79.99; H, 8.86; N, 3.66.

13-*cis*-*N*-(2-Hydroxyethyl)retinamide (IVd)—*From Isolated IIIc*—A solution of 5 g (14.3 mmol) of 1-(13-*cis*-retinoyl)imidazole (IIIc) in 50 mL of dry benzene was added slowly to a stirring mixture of 6.4 mL (105 mmol) of ethanolamine and 50 mL of benzene at 5–10°C. The mixture was stirred vigorously at 5°C for 2 h and then diluted with cold water (50 mL). Ether (100 mL) was added to cause separation of the emulsion. The benzene–ether layer was separated, washed with saturated aqueous sodium chloride, dried (Na_2SO_4), and concentrated under reduced pressure to a viscous syrup. The syrup was dissolved in hexane (50 mL) at room temperature, and the solution was filtered and stored at 5°C. A yellow, crystalline precipitate was collected by filtration and dried to give 3.5 g (71%). The crystalline product was only sparingly soluble in hexane, but it could be recrystallized by dissolving it in hot cyclohexane and cooling: yield 2.8 g (57%) of IVd; mp 107–109°C (cap., inserted at 95°C); HPLC (column B, solvent B): 98.8%.

From IIIc In Situ—A mixture of 13-*cis*-retinoic acid (50 g), 1,1′-carbonyldiimidazole (31 g), and dry benzene (500 mL) was stirred for 3 h at 20–25°C and then heated to 50°C for 15 min. The dark orange solution was slowly added to a stirring mixture of ethanolamine (62 mL) in benzene (500 mL) at ~10°C. This mixture was stirred for 2 h at 10–20°C; at this time TLC showed the absence of starting material. Cold, saturated, aqueous sodium chloride solution (500 mL) and then ether (500 mL) were added to the stirring

reaction mixture. The organic layer was separated and washed again with saturated aqueous NaCl. The benzene–ether solution was dried (Na_2SO_4) and concentrated *in vacuo* to a viscous syrup that was dissolved in hexane (450 mL) and refrigerated overnight. A yellow, crystalline precipitate was separated by filtration, slurried with hot hexane (400 mL) in which it was only slightly soluble, and recrystallized from cyclohexane to yield 38 g (66%) of IVd, mp 107–109°C (cap., inserted at 80°C); TLC: major spot and two slower, trace impurities (95:9 chloroform–methanol); HPLC (column B, solvent B): 99% IVd, retention time 6.6 min; 0.5% impurity, retention time 5.1 min; and 0.5% impurity, retention time 8 min.

Anal.—Calc. for $C_{22}H_{33}NO_2$: C, 76.93; H, 9.68; N, 4.08. Found: C, 76.82; H, 9.65; N, 3.94.

13-cis-*N*-(4-Hydroxybutyl)retinamide (IVe)—Compound IVe was prepared from 35 g (117 mmol) of IIIa, 21.7 g (134 mmol) of 1,1′-carbonyldiimidazole, and 350 mL of benzene by the second procedure described for the preparation of IVd, but the reaction mixture was concentrated under reduced pressure to a viscous syrup without first adding water. An ether (500 mL) solution of the syrup was extracted with water (2 × 250 mL), dried (Na_2SO_4), and evaporated to a viscous, orange syrup. A solution of the syrup in warm acetonitrile (200 mL) was filtered, diluted with warm water (~50 mL), seeded with IVe previously induced to crystallize from acetonitrile–water, cooled (with scratching) to room temperature, and stored in a refrigerator overnight. The pale yellow, crystalline precipitate was collected by filtration, washed sparingly with acetonitrile–water (2:1), and dried *in vacuo* to yield 26 g (60%) of IVe; mp 53–57°C (cap., inserted at 45°C); TLC: 1 spot with streaking (95:5 chloroform–methanol); HPLC (column B, solvent B): 99.3% IVe (retention time 6.8 min); 0.2% impurity, retention time 5.3 min; and 0.3% impurity, retention time 8.4 min.

Anal.—Calc. for $C_{24}H_{37}NO_2 \cdot 0.5H_2O$: C, 75.76; H, 10.07; N, 3.68. Found: C, 75.82; H, 10.08; N, 3.56.

Compound IVe was first obtained as a solvate with cyclohexane. The syrup obtained by the procedure described above from the ether solution was dissolved in warm cyclohexane. The solution was cooled to room temperature, refrigerated, and filtered to remove a yellow, crystalline product, mp 71–76°C (63% yield calculated as a 1:1 solvate). The solvate was recrystallized from cyclohexane (92% recovery) mp 73–76°C (cap., inserted at 65°C); TLC: IVe as a strong spot and a trace impurity (95:5 chloroform–methanol); HPLC: 99%. ^{1}H-NMR (δ 1.42, s, $CDCl_3$) and mass spectra (*m/z* 284) revealed the presence of cyclohexane; elemental analysis, UV, and ^{1}H-NMR indicated that the molar ratio of cyclohexane to IVe was ~1:1.

Anal.—Calc. for $C_{24}H_{37}NO_2 \cdot C_6H_{12}$: C, 79.08; H, 10.83; N, 3.08. Found: C, 78.87; H, 10.86; N, 3.00.

A specimen (43 g) kept in a high vacuum (continuous pumping, oil pump) at room temperature for 5 d did not lose weight. A specimen recrystallized from 90% acetonitrile appeared to retain at least some of the cyclohexane (mp 54–75°C; MS, *m/z* 84). A solution of the solvate in ethanol was evaorated to dryness *in vacuo*, and this process was repeated four times. The residual syrup was crystallized from acetonitrile–water, mp 53–57°C; HPLC: 98%. ^{1}H-NMR, UV, and MS indicated that the cyclohexane had been removed.

Stability and Storage—Observations of the sensitivity of several all-*trans*-retinamides in the crystalline state to atmospheric oxygen indicated that there are differences among compounds with this structure. The 2,3-dihydroxypropyl amide (IIi, polymorph with mp 83–85°C) appeared to be the most susceptible to deterioration during deliberate exposure to air. The crystalline material became amorphous, and the UV maximum decreased markedly. Eventually, the UV spectrum became a broad plateau with very low absorbance from 220–360 nm and slight elevations centered near 340 and 240 nm. HPLC analyses monitored by UV and based on relative areas may give grossly inaccurate (and high) assays of retinoids that have deteriorated in this manner. However, IIi could be stored for long periods in a liquid-nitrogen freezer, at low temperatures in flame-sealed ampules containing an inert atmosphere, or at low temperatures in heat-sealed, impervious plastic bags (below) containing an inert atmosphere. Crystalline specimens of the 2-hydroxyethyl (IId), 2-hydroxypropyl (IIf), and 4-hydroxybutyl (IIh) all-*trans* amides were also susceptible, but less so, to absorption of atmospheric oxygen; the ethyl (IIb) and 4-hydroxyphenyl (IIm) amides appeared to be more stable. These differences probably arise from differences in crystal form and particle size. For example, as expected, a finely crushed specimen of IIi (lower-melting polymorph) absorbed oxygen more rapidly than did an uncrushed specimen; also, the lower-melting polymorph of IIi absorbed oxygen more rapidly than did the higher-melting polymorph. Storage of retinoids at very low temperatures does not necessarily prevent deterioration unless the retinoids are continuously in an inert atmosphere such as that provided by a liquid-nitrogen freezer, flame-sealed ampules containing nitrogen or argon, or heat-sealed plastic bags containing argon or nitrogen. If a retinoid is placed in a container sealed at room temperature with a screw cap and if the container contracts more than the cap, crevices may develop at low temperatures that would permit the exchange of outside air with the container atmosphere.

Crystalline specimens of the retinamides (II and IV) were stored in an argon atmosphere in flame-sealed, all-glass ampules or in a liquid-nitrogen freezer. The ampules were the freeze-drying type, so they could withstand evacuation with a vacuum pump. The retinamide were put into ampules in a nitrogen atmosphere in a controlled-atmosphere box. After the ampules were loaded with the retinoids, the ampule necks were cleared of adhering crystals to prevent decomposition of small amounts of the retinoids during flame-sealing. The ampules were then attached to a system that included a manometer and a vacuum pump and evacuated; argon was then admitted into the ampules to atmospheric pressure. This process was repeated five times. After the final admission of argon, the neck of the ampule (still attached to the closed system) was sealed with a torch while the body of the ampule containing the retinoid was kept cool. The seals of the ampules were examined with a stereoscope (magnification 30×). The ampules were then placed in dry ice or in a freezer at −80°C at least overnight. The seals were then reexamined with the stereoscope to see if cracks had developed because of stresses during storage at low temperatures. If the retinoid was to be stored for extended periods, the ampules were kept either at −20°C or at −80°C. Retinoids stored for short periods of time were put in brown, screw-cap bottles. Argon or nitrogen was admitted into these bottles in an evacuated desiccator. Each sealed bottle, with tape around the cap, was then placed in an impervious plastic bag. The bag was heat-sealed except for a hole in a corner, flushed with nitrogen or argon with a tube through the corner, and then sealed completely. The sealing was done with a heat-pressure device.

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Analysis of Oxprenolol in Formulations by High-Performance Liquid Chromatography

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Abstract □ A simple, accurate, and rapid high-performance liquid chromatographic method for the analysis of oxprenolol in commercial formulations is described. The analysis was performed on a cyano radial compression cartridge, with 0.0539 M, pH 3 phosphate buffer–acetonitrile–methanol (76:15.6:8.4) as the mobile phase. The flow rate was 5 mL/min, with detection at 272 nm; the mobile phase was employed for extraction. The assay was applied to the content uniformity test of three oxprenolol hydrochloride tablet formulations of different strengths and the contents of a 2-mg dry ampule for intravenous/intramuscular injection. The percent of label claim for each formulation tested was within 91.7–110%. The applicability of this assay to the analysis of some other β-blocking drugs was investigated. It was found that under the above conditions, atenolol, metoprolol, oxprenolol, and propranolol can be fully resolved in <3 min.

Keyphrases □ Oxprenolol—tablet and dry ampule formulations, assay alone or simultaneously with other β-adrenergic drugs, HPLC □ β-Adrenergic drugs—oxprenolol, propranolol, atenolol, and metoprolol, individual and simultaneous assay in formulations, HPLC

Oxprenolol, (±)-1-[*o*-(allyloxy)phenoxy]-3-(isopropylamino)-2-propanol (I), is a β-adrenergic drug frequently used in the treatment of hypertension (1), cardiac arrhythmias (2), and angina pectoris (3). The low dosage of I, particularly when administered intravenously, and its susceptibility to light and other storage conditions require assurance of potency and content uniformity of its dosage forms.

Several methods have been reported for the analysis of I in biological fluids including GC (4–8), TLC with fluorescence detection (9), and high-performance liquid chromatography (HPLC) (10, 11). While the GC assays involve prechromatography derivatization and multiple-step extraction, the TLC method includes derivatization of I to a fluorescing compound. The utilization of HPLC for the analysis of I provides a real advantage, since it does not involve lengthy derivatization steps and the compound is detected directly. However, the previously reported HPLC methods, in addition to lacking a high chromatographic efficiency, have not been applied to the analysis of I in formulations. No pharmacopeial assay of I is available.

The present report describes an expedient, accurate, and specific HPLC method for the analysis of I in formulations. The procedure can be applied for the chromatography of some other β-blocking drugs (atenolol, metoprolol, nadolol, and propranolol) under the same conditions employed for I.

EXPERIMENTAL

Materials—Methanol[1], acetonitrile[1], hexane[2], monobasic sodium phosphate[2], and 85% phosphoric acid[2] were either analytical or HPLC grade. Purified water[3] was used as obtained. The oxprenolol hydrochloride formulations[4] tested (20-, 40-, and 80-mg tablets and 2-mg dry ampules) were obtained in-house. Propranolol hydrochloride[5], nadolol[6], atenolol[5], and metoprolol tartrate[7] were used as received. Oxprenolol (I) was first extracted from a saturated solution of oxprenolol hydrochloride in 5 M NaOH with boiling hexane and was recrystallized several times (mp 77.1°C) from hexane.

Apparatus—The chromatograph used consisted of dual solvent delivery systems[8] with a mixing chamber, automatic sample injection processor[9], printer-plotter integrator data module[10], variable-wavelength UV detector[11], and radial compression module[12]. The analysis was performed on a 10-μm, 8-mm × 10-cm cartridge[13].

Chromatography Conditions—The mobile phase was methanol–acetonitrile–0.0539 M sodium phosphate buffer solution at pH 3 (8.4:15.6:76) filtered twice and deaerated before use. A flow rate of 5 mL/min was used throughout (pressure = 1100 psi). The UV detector was set at 272 nm, and the volume injected into the cyano cartridge was 40–60 μL.

[1] Burdick and Jackson Laboratories Inc., Muskegon, Mich.
[2] Fisher Scientific Company, Fair Lawn, N.J.
[3] Du Pont, Wilmington, Del.
[4] Ciba Laboratories, Horsham, Switzerland.
[5] ICI Limited, Macclesfield, Cheshire, U.K.
[6] E. R. Squibb & Sons, New Brunswick, N.J.
[7] Ciba-Geigy Co., Basle, Switzerland.
[8] Model M45; Waters Associates, Milford, Mass.
[9] Model 710B WISP; Waters Associates, Milford, Mass.
[10] Model 730 Data Module; Waters Associates, Milford, Mass.
[11] Model 480 Lambda; Waters Associates, Milford, Mass.
[12] Model RCM-100; Waters Associates, Milford, Mass.
[13] Radial Pak μ Bondapak CN; Waters Associates, Milford, Mass.

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