N-(Retinoyl)amino Acids. Synthesis and Chemopreventive Activity in Vitro

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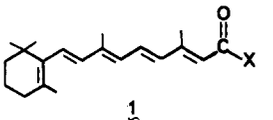
N-(*all-trans*-Retinoyl)amino acids were synthesized via *all-trans*-retinoyl chloride and an ester of the amino acid. The retinoyl derivatives of leucine, phenylalanine, alanine, tyrosine, and glutamic acid were prepared. The 13-*cis*-retinoyl derivatives of leucine, phenylalanine, alanine, and glycine were prepared similarly from 13-*cis*-retinoic acid. In assays of the retinoylamino acids for reversal of squamous metaplasia in hamster trachea organ cultures, these compounds were less active than retinoic acid, but the leucine, alanine, and phenylalanine derivatives were similar in activity to several retinamides that suppress bladder carcinogenesis in vivo. Two of the retinoylamino acids, as well as two simple retinamides, were shown to be moderately cytotoxic to murine leukemia and human epidermoid carcinoma cells in culture.

Numerous studies of vitamin A and vitamin A deficiencies have shown that compounds of the vitamin A group are essential for the differentiation and proliferation of epithelial cells and for the growth and maintenance of epithelial tissues.[1-6] Vitamin A deficiency in animals evokes pathological changes in many organs and tissues,[1,2,7] and various organs or tissues cultured in retinoid-free media or in the presence of carcinogens develop potentially premalignant lesions (hyperplasia or squamous metaplasia).[8-14] Certain retinoids[15] have the capacity to prevent or reverse these deficiency-produced or carcinogen-induced epithelial lesions[4,8-14] or to induce differentiation of malignant cells in culture.[16,17] Furthermore, administration of certain retinoids to animals can prevent or reduce the incidence of carcinogen-induced neoplasia in vivo.[3,4,18-26] However, the usefulness of natural retinoids for chemoprevention[15] is limited by their toxicity.[3,20] Certain synthetic retinoids that possess chemopreventive activity are less toxic than the natural retinoids[18,22,23] and produce higher retinoid concentrations in certain target organs (e.g., the mammary gland[11,27]). Many of the retinoids that have demonstrated chemopreventive activity in organ-culture assays and in vivo are derivatives or analogues of *all-trans*-retinoic acid (**1a**). Retinamides (e.g., **1c–e** and **2c–e**) are prominent among derivatives that have been shown to reduce the incidence of carcinogen-induced cancers in vivo, e.g., in the bladder,[22-24] mammary gland,[24,27] and pancreas.[25]



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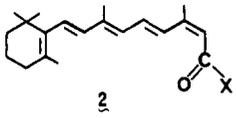

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a: X = OH (all-*trans*-RA, **1a**; 13-*cis*-RA, **2a**)

b: X = Cl **d**: X = $-NHCH_2CH_2OH$

c: X = $-NHC_2H_5$ **e**: X = $-NHC_6H_4OH-4$

N-(*all-trans*-Retinoyl)amino acids (e.g., **6**) and *N*-(13-*cis*-retinoyl)amino acids (e.g., **16**), which are amides of the corresponding retinoic acids, provide a way of altering polarity at the terminal end of the retinoid structure. Certain advantages may be postulated a priori for this type of retinamide. Water solubility should be increased in comparison with the solubility of simple alkyl or aryl amides. Increased water solubility might be advantageous for the suppression of carcinogenesis at some sites such as the bladder. Obviously, water solubility will vary as the

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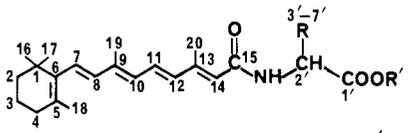
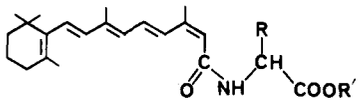
size of the R group changes, as in the glycine (**3**) and the leucine (**6**) derivatives, or as the R group changes in polarity, as in the leucine (**6**) and the glutamic acid (**14**) derivatives. Furthermore, retinoylamino acids may be regarded formally as homologues of retinoic acid in which the –NHCH(R)– group extends the side chain. Since these derivatives have a free carboxyl group, they might bind to cellular retinoic acid binding protein (CRABP). Also, we had synthesized *N*-(*all-trans*-retinoyl)glycine (**3**) earlier.[28,29] Sporn and co-workers found that **3** is active in the hamster trachea organ-culture assay[30] and is a strong inducer of RA metabolism,[31] and Chopra and Wilkoff[32] reported that it is more active against benzo[*a*]pyrene-induced hyperplasia in mouse prostate cultures than is RA. For these several reasons, representative *N*-(*all-trans*-retinoyl)amino acids (**6**, **8**, **10**, **12**, **14**) and *N*-(13-*cis*-retinoyl)amino acids (**16**, **18**, **20**, **22**) were prepared. The syntheses and initial bioassays of these retinoids are summarized herein.

The (*all-trans*-retinoyl)amino acids were prepared from *all-trans*-RA (**1a**) via retinoyl chloride (**1b**). A benzene solution of **1b**, prepared in situ,[28] was added to a solution of an ester of the L- or DL-amino acid hydrochloride and triethylamine in dimethylformamide. After the retinoylamino ester had been purified by chromatography on silica gel, it was hydrolyzed in a solution of potassium hydroxide in ethanol, and the amino acid was precipitated from an aqueous solution at pH 3.5. *N*-(13-*cis*-Retinoyl)amino acids (**16**, **18**, **20**, **22**) were prepared similarly from 13-*cis*-retinoic acid (**2a**) via 13-*cis*-retinoyl chloride[28] (**2b**). The hydrolysis step was performed at ambient temperatures in 90% ethanol, and TLC of aliquot portions of the reaction solution was employed to estimate the time required. The reaction solution was then diluted with water and neutralized before it was concentrated to remove ethanol; the concentrated aqueous solution was acidified to pH 3.5 to precipitate the retinoylamino acid. If the hydrolysis of an L-amino acid ester was performed at 50–60 °C or if the basic reaction solution was concentrated to remove most of the ethanol without first neutralizing the excess base, considerable racemization occurred. Recently, the preparation of the glycine derivative[28] (**3**), *N*-retinoyl-DL-phenylalanine, and N^{α}-retinoyl-L-lysine from retinoyl fluoride was reported.[33]

In the proton NMR spectra of the four pairs of *all-trans*- and 13-*cis*-retinoylamino acids, the major differences between the all-trans and 13-cis isomers are, as expected, the pronounced downfield shift of the signal from the proton at position 12 and the smaller upfield shifts of the signals



	R	R′
3	H	H
4	$(CH_3)_2CHCH_2$–	C_2H_5
5	$(CH_3)_2CHCH_2$–	CH_3
6	$(CH_3)_2CHCH_2$–	H
7	$C_6H_5CH_2$–	CH_3
8	$C_6H_5CH_2$–	H
9	CH_3	C_2H_5
10	CH_3	H
11	4–HO–$C_6H_4CH_2$–	C_2H_5
12	4–HO–$C_6H_4CH_2$–	H
13	$C_2H_5OOCCH_2CH_2$–	C_2H_5
14	$HOOCCH_2CH_2$–	H



	R	R′
15	$(CH_3)_2CHCH_2$–	C_2H_5
16	$(CH_3)_2CHCH_2$–	H
17	$C_6H_5CH_2$–	CH_3
18	$C_6H_5CH_2$–	H
19	CH_3	C_2H_5
20	CH_3	H
21	H	C_2H_5
22	H	H

from the protons at positions 14 and 20 in the spectra of the 13-cis isomers. The signals arising from the protons at positions 12, 14, and 20 appear respectively at δ 6.24, 5.72, and 2.36 in the spectra of the all-trans leucine (**6**) and alanine (**10**) derivatives (in deuteriated chloroform), whereas the corresponding chemical shifts in the spectra of the 13-cis leucine (**16**) and alanine (**20**) derivatives appear respectively at δ 7.78, 5.58, and 2.03. The differences between the chemical shifts of these sets of protons in the spectra of the glycine (**3** and **22**) and phenylalanine (**8** and **18**) derivatives are similar. In contrast, the chemical shifts of the protons at the other proximal positions, 11 and 2′, were similar in the spectra of the pairs of all-trans and 13-cis isomers, except for the glycine derivatives.

The electron-impact mass spectra of all of the retinoylamino acids and of three esters (**4**, **11**, **21**) were determined, and all included the following peaks (M = the molecular ion): *m/z* M, M + H, M – CH_3, 282 [M – NHCH(R)COOR′ – H], 267 [282 – CH_3], 255 [M – CONH(R)COOR′], 240 [center of 3-peak cluster, M – CH_3 – CONHCH(R)COOR′], 255 [240 – CH_3]. Most of the spectra also included peaks corresponding to M – OR′, M – 2,6,6-trimethylcyclohexenyl, and 298 [M – CH(R)-COOR′].

Bioassays. The retinoylamino acids were evaluated in the hamster trachea organ culture assay developed by Sporn and co-workers.[10–12] This bioassay is specific for retinoids[12] and assesses the capacity of retinoids to reverse keratinization in vitamin A deficient hamster trachea in culture. Therefore, it is a measure of the capacity of a retinoid to control differentiation of epithelial cells, and it is believed to have significant predictive value for the potential of a retinoid to prevent epithelial cancers.[12]

The results of assays of the retinoylamino acids in the hamster-trachea system are summarized in Table I.

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all-trans-Retinoic acid was assayed as a positive-control retinoid in each experiment. Also, data obtained by Newton et al.[12] in assays of six *all-trans*- or 13-*cis*-retinamides, as well as historical values[12] of ED_{50} for many assays of *all-trans*-RA and 13-*cis*-RA, are included in Table I for comparison. These six retinamides,[23,24] as well as 13-*cis*-RA,[19] suppress bladder carcinogenesis in vivo. The data show that none of the amino acid derivatives were as active as *all-trans*-RA, but the leucine, alanine, and phenylalanine derivatives appeared to be comparable, at least, in activity to the more active of the simpler retinamides. *N*-(*all-trans*-Retinoyl)leucine (**6**) may be somewhat more active than the other members of this group. The glutamic acid derivative **14**, the most polar of the retinoylamino acids, was the least active in this bioassay. The tyrosine derivative **12** also appeared to be less active than the less polar leucine, phenylalanine, and alanine derivatives.[34] The activities of the retinoylamino acids in the hamster-trachea system indicate that further studies, in vitro and in vivo, of the chemopreventive potential of these retinoids are warranted.

N-(*all-trans*-Retinoyl)glycine (**3**) and *N*-(*all-trans*-retinoyl)leucine (**6**) were tested for inhibition of the proliferation of two kinds of neoplastic cells in culture. The results of these tests, together with tests of the 13-*cis*-*N*-ethyl (**2c**) and 13-*cis*-*N*-(2-hydroxyethyl) (**2d**) retinamides, are summarized in Table II. All four of these compounds inhibited proliferation of L1210 cells at 20 and 40 μg/mL; the two retinoylamino acids appear to be somewhat more active than the two simple amides. In addition, all four of these retinoids were active against human epidermoid carcinoma (H.Ep.-2) cells at 20 and 40 μg/mL, and *trans*-retinoylleucine (**6**), as well as **2c** and **2d**, displayed significant activity at 10 μg/mL.

Experimental Section

General Methods. All operations involved in the preparation, isolation, purification, and transfer of retinoids were performed in an atmosphere, or under a current, of 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 or −80 °C.

Melting temperatures were determined in capillary tubes heated in a Mel-Temp apparatus. Ultraviolet spectra (UV) were determined with ethanol solutions and were recorded with a Cary Model 17 spectrophotometer; maxima are given in nanometers. Infrared spectra (IR) were determined from specimens in pressed potassium bromide disks, unless indicated otherwise, and were recorded with a Nicolet Model MX-10 Fourier transform IR spectrometer; s = strong, w = weak, br = broad, sh = shoulder. Mass spectral (MS) data were taken from low-resolution, electron-impact spectra determined at 70 eV with a Varian/MAT Model 311A spectrometer. Proton nuclear magnetic resonance spectra (^{1}H NMR) were determined at 100.1 MHz with a Varian XL-100-15 spectrometer or at 300.64 MHz with a Nicolet 300 NB NMR spectrometer; tetramethylsilane was the internal standard. Assignments of chemical shifts are designated by the position numbers shown on the general (*all-trans*-retinoyl)amino acid (or ester) structure representing **3**–**14**. Multiplicity and the position numbers are given parenthetically with each chemical shift; s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Thin-layer chromatography (TLC) was performed on plates of fluorescing silica gel, and developed plates were examined with UV lamps (254 and 365 nm). High-pressure liquid chromatography (HPLC) was performed with Waters Associates components systems and a Hewlett-Packard Model 3380-S integrator or with a Hewlett-Packard Model 1084B system. HPLC was performed on columns packed with octadecylsilylated silica (Spherisorb ODS), 5-μm particle size; unless indicated otherwise, the eluting solvent was 85:15 acetonitrile–1% aqueous ammonium acetate, isocratic, 1 mL/min flow rate; and elution was monitored by UV absorption at 340 nm. Optical rotations were determined at 546 nm (a mercury line) and at 589 nm (sodium D line) with an automatic polarimeter (Perkin-Elmer Model 241).

***N*-(*all-trans*-Retinoyl)glycine (3)** and the precursor ethyl ester were prepared by procedures described previously,[28] and **3** was recrystallized from acetonitrile: mp 104–106 °C (inserted at 60 °C); HPLC (70:30 acetonitrile–1% aqueous ammonium acetate), 99.4–99.8%; UV (ethanol) 348 nm (ϵ 49 800); ^{1}H NMR (300.64 MHz, $CDCl_3$) δ 1.03 (s, C16 + C17), 1.48 (m, C2), 1.61 (m, C3), 1.72 (s, C18), 1.99 (s, C19), 2.02 (m, C4), 2.34 (s, C20), 4.12 (d, C2′), 5.77 (s, C14), 6.12 (m, C7, C10), 6.24 (m, C12), 6.27 (m, C8), 6.49 (NH), 6.95 (d of d, C11), 10.37 m (COOH). A different crystal form was obtained earlier:[28] mp 94–97 °C; data from ^{1}H NMR and ^{13}C NMR spectra at 100.6 and 25.2 MHz, respectively, and other physical data have been reported.[29]

***N*-(*all-trans*-Retinoyl)-L-leucine Ethyl Eser (4).** A solution of 2.95 g (21.5 mmol) of phosphorus trichloride in 15 mL of dry benzene was added slowly to a stirring suspension of 9 g (30 mmol) of *all-trans*-retinoic acid (**1a**) in 105 mL of dry benzene. Stirring was continued for 1 h after the mixture became homogeneous (ca. 3 h).[28] A solution of L-leucine ethyl ester was obtained by dissolving 12 g (61 mmol) of L-leucine ethyl ester hydrochloride in 60 mL of dry dimethylformamide, adding 12.6 mL (91 mmol) of triethylamine, stirring the mixture for 5 min, filtering the mixture to remove the triethylamine hydrochloride, and cooling the filtate to 10 °C. To the cold stirring filtrate was slowly added the decanted[28] benzene solution of *all-trans*-retinoyl chloride (**1b**). The reaction mixture was stirred for 1 h and washed twice with water and once with sodium chloride solution, and the organic layer was dried over magnesium sulfate and concentrated to an orange syrup. This material was purified by chromatography on a column of silica gel 60 with chloroform as the eluting solvent. Fractions containing **4** were identified by TLC, combined, and concentrated in vacuo to a syrup. Evaporation of ethanol from the syrup left a foam: wt, 12 g; HPLC, 98.8%; $[\alpha]^{25}_{589}$ +37.5° and $[\alpha]^{25}_{546}$ +52.6° (*c* 0.8 in DMF); UV_{max} 348 nm (ϵ 45 900); ^{1}H NMR (300.64 MHz, $CDCl_3$) δ 0.96 (m, 5′ + 6′), 1.03 (s, 16 + 17), 1.28 (t, ester CH_3), 1.47 (m, 2), 1.62 (m, 3, 3′, 4′), 1.71 (s, 18), 1.97 (s, 19), 2.02 (m, 4), 2.34 (s, 20), 4.19 (q, ester CH_2), 4.69 (m, 2′), 5.72 (s, 14), 5.94 (d, NH), 6.13 (m, 8 or 7, 10), 6.23 (m, 7 or 8), 6.25 (m, 12), 6.93 (d of d, C11). Anal. ($C_{28}H_{43}NO_3 \cdot {}^1/_8H_2O$) C, H, N.

***N*-(*all-trans*-Retinoyl)-DL-leucine methyl ester (5)** was prepared from *all-trans*-retinoic acid (3 g, 10 mmol) and DL-leucine methyl ester hydrochloride (3.6 g, 20 mmol) by the procedure described for the preparation of **4**. After the reaction mixture had been stirred at 10 °C for 1 h, cold water (100 mL) was introduced into the stirring mixture, and ether (100 mL) was added to accelerate separation of the emulsion. The organic layer was washed with water (2×) and with sodium chloride solution, dried ($MgSO_4$), and concentrated to a syrup that was purified by column chromatography on silica gel 60 as described for **4**: wt, 4 g; HPLC (90:10 acetonitrile–1% aqueous ammonium acetate), 98.7%. This material was hydrolyzed to DL-**6** (below).

***N*-(*all-trans*-Retinoyl)leucine (6).** Potassium hydroxide (pellets, 0.7 g of 87% KOH, 10.9 mmol) was added to a solution of 4 g (9.3 mmol) of **5** in 40 mL of 95% ethanol, and the solution was stirred at room temperature for 3 h. (Aliquot portions were examined by TLC at half-hour intervals to monitor the disap-

(34) The validity of a comparison of the activity of a retinoyl-DL-amino acid (e.g., **6**) with that of a retinoyl-L-amino acid (e.g., **18**) might be questioned. If one assumes that *only* the L enantiomer of a DL derivative is active, the concentration of the active component is half of the concentration listed in Table I. For example, the values of ED_{50} of the supposed active component (L) of the *trans*-Leu (**6**) and the *cis*-Phe (**18**) derivatives would be 5×10^{-11} and 3×10^{-10}, respectively. These differences from the values (Table I) of 1×10^{-10} (calculated from three experiments) and 6×10^{-10} (calculated from two experiments) are within the boundaries of variation of assay results from different experiments based on seven or eight tracheas. For example, the individual values of ED_{50} for the three assays of *trans*-Leu (**6**) were 1×10^{-10}, 8×10^{-11}, and 2×10^{-10}, and the values for the two assays of *cis*-Phe (**20**) were 1×10^{-9} and 3×10^{-10}.

Table I. *N*-(Retinoyl)amino Acids: Evaluation in the Hamster Trachea Organ Culture Assay[a]

	reversal of keratinization				% of cultures with different degrees of squamous metaplasia				
compd[b]	concn, M	no. of cultures[c] active/total	ED_{50} of test compd,[d] M	ED_{50} of RA,[e] M	none	minimal	mild	marked	severe
6 (*t*-Leu)[f]	10^{-8}	22/23	1×10^{-10}	1×10^{-11}	96	0	4	0	0
	10^{-9}	20/23			87	0	4	9	0
	10^{-10}	12/25			48	0	16	32	4
	10^{-11}	2/14			14	0	14	58	14
8 (*t*-Phe)[g]	10^{-8}	5/7	5×10^{-10}	8×10^{-12}	71	0	29	0	0
	10^{-9}	4/7			57	0	0	43	0
	10^{-10}	3/7			43	0	29	14	14
	10^{-11}	1/7			14	0	14	72	0
10 (*t*-Ala)[h]	10^{-8}	7/7	4×10^{-10}	1×10^{-11}	100	0	0	0	0
	10^{-9}	5/7			72	0	14	0	14
	10^{-10}	1/7			14	0	14	58	14
	10^{-11}	0/7			0	0	29	57	14
12 (*t*-Tyr)[f]	10^{-8}	6/8	3×10^{-9}	1×10^{-11}	75	0	0	25	0
	10^{-9}	3/8			38	0	12	50	0
	10^{-10}	1/7			14	0	14	58	14
14 (*t*-Glu)[f]	10^{-8}	3/8	$>10^{-8}$	2×10^{-11}	38	0	12	50	0
	10^{-9}	2/8			25	0	50	25	0
	10^{-10}	1/7			14	14	29	43	0
16 (*c*-Leu)[i]	10^{-8}	5/7	5×10^{-10}	8×10^{-12}	72	0	14	14	0
	10^{-9}	4/7			57	0	29	14	0
	10^{-10}	2/7			29	0	14	57	0
	10^{-11}	2/6			33	0	0	50	17
18 (*c*-Phe)[f]	10^{-8}	9/15	6×10^{-10}	1×10^{-11}	60	0	13	20	7
	10^{-9}	8/15			53	0	20	20	7
	10^{-10}	4/14			29	0	21	36	14
	10^{-11}	4/14			29	0	14	43	14
20 (*c*-Ala)[j]	10^{-8}	15/15	7×10^{-10}	1×10^{-11}	100	0	0	0	0
	10^{-9}	7/15			47	0	13	40	0
	10^{-10}	5/14			36	0	21	36	7
	10^{-11}	1/7			14	0	0	71	15
all-trans-*N*-ethyl-retinamide (**1c**)			1×10^{-9}						
all-trans-*N*-(2-hydroxyethyl)retinamide (**1d**)			1×10^{-10}						
all-trans-4-HPR (**1e**)			3×10^{-10}						
13-*cis*-*N*-ethylretinamide (**2c**)			3×10^{-10}						
13-*cis*-*N*-(2-hydroxyethyl)retinamide (**2d**)			3×10^{-10}						
13-*cis*-4-HPR (**2e**)			$>1 \times 10^{-9}$						
all-trans-RA (**1a**)			3×10^{-11}						
13-*cis*-RA (**2a**)			3×10^{-11}						

[a] The procedures for performing this bioassay are outlined and referenced in the Experimental Section. [b] The values of ED_{50} for **1a**,**c**–**d** and **2a**,**c**–**d** are data reported by Newton et al.[12] [c] The number of active cultures includes only cultures that showed no evidence of squamous metaplasia (column 6). It does not include partial inhibition or reversal of squamous metaplasia (columns 7–10). [d] These values of ED_{50} are calculated from the number of cultures that showed no evidence of squamous metaplasia (column 6). They do not include partial inhibition or reversal of squamous metaplasia (columns 7–10). [e] *All-trans*-Retinoic acid was the positive control retinoid. These values of ED_{50} were calculated from assays of *all-trans*-RA performed simultaneously with the assays of each new retinoid. [f] The specimen submitted for bioassays was the racemic form. [g] Essentially the racemic form (>90% DL; cf. that in the Experimental Section). [h] Mostly the racemic form (about $^3/_4$ DL; cf. that in the Experimental Section). [i] L form. [j] Mostly the L form (about $^1/_3$ DL; cf. that in the Experimental Section).

pearance of **6**; none was observable at 2.5 h.) Water (50 mL) was added, the pH of the solution was adjusted to 6 with dilute hydrochloric acid, the solution was concentrated in vacuo (to about 30 mL) to remove most of the ethanol, and more water (100 mL) was added. The pH of the resulting solution was lowered to 3.5 to precipitate DL-**6**, and the pale yellow solid was collected by filtration and dried: wt 3.7 g (95%); mp 175–176 °C. This material was dissolved in refluxing acetonitrile (110 mL), and a pale yellow precipitate (needles) was separated from the chilled (−20 °C) mixture: yield of DL-**6**, 2.8 g (72%); mp 178–180 °C (inserted at 165 °C); HPLC, 99.6% (retention time, 8.8 min); UV_{max} 346 nm (ϵ 52 000); ^1H NMR (300.64 MHz, $CDCl_3$) δ 0.97 (m, 5′ + 6′), 1.03 (s, 16 + 17), 1.48 (m, 2), 1.62 (m, 3 + 1 H of 3′), 1.72 (s, 18), 1.76 (m, 4′ + 1 H of 3′), 2.00 (s, 19), 2.04 (m, 4), 2.36 (s, 20), 4.64 (m, 2′), 5.73 (s, 14), 5.95 d (NH), 6.14 (m, 10 + 8 or 7), 6.24 (m, 12), 6.27 (m, 7 or 8), 6.97 (d of d, 11), 10.55 (br, COOH). Anal. ($C_{26}H_{39}NO_3$) C, H, N.

A specimen of the L-leucine ethyl ester (**4**) was hydrolyzed similarly to crude L-**6**: yield, 89%; mp 74–78 °C (capillary inserted at 60 °C); HPLC, 99.5%; $[\alpha]^{25}_{589}$ +53.3° and $[\alpha]^{25}_{546}$ +71.9° (*c* 0.93 in DMF). This material was not purified further.

Earlier, in several experiments **4** was hydrolyzed at higher temperatures (e.g., 50 °C) in potassium hydroxide–absolute ethanol solutions, and the reaction mixtures were diluted with water and concentrated without first neutralizing the solution. Under these reaction and workup conditions, considerable racemization occurred. The initial precipitates obtained by acidification of the diluted reaction solutions were obtained in high yields usually, but were either amorphous or were low-melting solids. Solid products would usually melt in the range 70–80 °C, resolidify, and remelt above 160 °C. Trituration of the initial precipitates with acetonitrile and then crystallization of the undissolved solids from acetonitrile–water afforded DL-**6** in low or modest yields; mp 178–180 °C. One such hydrolysis of **4** furnished a quantitative yield of the initial precipitate: HPLC, 98.7%; $[\alpha]^{23}_{589}$ +20.5° (*c* 0.3 in DMF). After acetonitrile was used to triturate and then recrystallize the crude product, DL-**6** was obtained in 31% yield; mp 178–179 °C; HPLC, 99.6%. The filtrate from the trituration furnished a residue with $[\alpha]^{25}_{589}$ +39.7° and $[\alpha]^{25}_{546}$ +54.4° (c 1.26 in DMF).

***N*-(*all-trans*-Retinoyl)phenylalanine Methyl Ester (7).** A solution of L-phenylalanine methyl ester was prepared from 4.3 g (20 mmol) of the hydrochloride, 4.2 mL (30 mmol) of triethylamine, and 25 mL of anhydrous DMF. The mixture was stirred for 5 min, filtered to remove triethylamine hydrochloride, and cooled to 10 °C. A solution of retinoyl chloride in benzene was prepared, as outlined above (for **4**) and as described previously,[28] from 3 g (10 mmol) of *all-trans*-retinoic acid and 35 mL

Table II. Cytotoxicity to Neoplastic Cells in Culture

compd	concn, μg/mL	proliferation of L1210 cells, % of control[a]	viability of H.Ep.-2 cells, % of control[b]
2c	1	84	59
	10	77	13
	20	54	10
	40	*c*	15
3 (*t*-Gly)	1	68	116
	10	70	67
	20	19	12
	40	*c*	13
2d	1	76	61
	10	57	38
	20	56	21
	40	0.5	27
6 (*t*-Leu)	1	95	73
	10	62	29
	20	20	14
	40	*c*	25

[a] This quantity is a measure of inhibition of *proliferation* of L1210 cells in culture. The compound was added to a suspension of a determined number of L1210 cells (about 10^5) in culture. After incubation of the cell culture at 37 °C for 48 h, the number of cells was redetermined. At each concentration of the retinoid, the number of cells at 48 h was the average of the cell counts of three cultures. Percent of control = 100 × [(no. of cells at 48 h − no. of cells at 0 h)/(no. of control cells at 48 h − no. of control cells at 0 h)]. [b] This quantity is a measure of the effect of a compound on the viability of human epidermoid carcinoma cells (H.Ep.-2) by the clone-colony technique. Cultures were incubated at 37 °C for 12 days and, after staining, colonies were counted. Percent of control = 100 × (no. of colonies in treated cultures/no. of colonies in control cultures). [c] The number of cells after 48 h was less than the number of cells at 0 h.

of benzene and was added dropwise to the cold amino ester solution. The mixture was stirred for 1 h and then washed twice with cold water and once with sodium chloride solution. The organic layer was dried with magnesium sulfate and concentrated in vacuo to an orange syrup. The crude product was chromatographed on a column of silica gel 60; chloroform was the eluting solvent. Product-containing fractions were identified by TLC and were combined, and the total solution was concentrated in vacuo to a syrup: yield of L-**7**, 4.1 g (89%); HPLC (85:15 acetonitrile–water), 99.6%; $[\alpha]^{25}_{589}$ −64.8° and $[\alpha]^{25}_{546}$ −85.1° (c 1.18 in DMF).

***N*-(*all-trans*-Retinoyl)phenylalanine (8).** Potassium hydroxide (pellets, 0.7 g of 87% KOH, 10.9 mmol) was added to a solution of 4.2 g (9.1 mmol) of the methyl ester (L-**7**) in 50 mL of 90% ethanol, and the solution was stirred at room temperature for 1 h. (The starting material was not observable by TLC of an aliquot portion removed after 0.5 h.) Cold water (50 mL) was added, the pH of the resulting solution was adjusted to 7 with dilute hydrochloric acid, the solution was concentrated in vacuo to remove most of the ethanol, and more water (150 mL) was added. The pH of the resulting aqueous solution was lowered to 3.5 by adding dilute hydrochloric acid, and the yellow precipitate was collected by filtration and dried in vacuo: wt, 3.7 g (91%); HPLC, 100%. A solution of this material in hot acetonitrile (30 mL) was diluted with water, cooled to room temperature, and stored overnight at 5 °C. The pale yellow precipitate (L-**8**) was collected by filtration and dried in vacuo: yield, 2.6 g (64%); mp 141–143 °C (inserted at 130 °C); HPLC, 100%; $[\alpha]^{25}_{589}$ −81.2° and $[\alpha]^{25}_{546}$ −108° (*c* 1.05 in DMF); UV_{max} 346 nm (ε 51 900); ^1H NMR (300.64 MHz, $CDCl_3$) δ 1.02 (s, 16 + 17), 1.47 (m, 2), 1.62 (m, 3), 1.71 (s, 18), 1.99 (s, 19), 2.02 (m, 4), 2.31 (s, 20), 3.17 (m, 3′), 3.26 (m, 3′), 4.92 (m, 2′), 5.64 (s, 14), 6.00 (d, NH), 6.11 (m, 10), 6.13 (m, 8 or 7), 6.20 (m, 12), 6.27 (m, 7 or 8), 6.94 (d of d, 11), 7.10–7.35 (m, phenyl), 11.02 (br, COOH). Anal. ($C_{29}H_{37}NO_3$) C, H, N.

When **7** was treated with potassium hydroxide in absolute ethanol and the water-diluted reaction mixture was concentrated without first neutralizing the alkaline solution, racemization occurred (cf. **6**). The initial low-melting precipitate (70% yield) was recrystallized from acetonitrile–water: yield of yellow crystalline solid, 27%; mp 152–153 °C; HPLC, 99.7%; $[\alpha]^{23}_{589}$ −6.3 (c 1 in DMF). This material was used in bioassays in vitro; the value of the optical rotation compared to that of L-**8** above indicated that this specimen was >90% DL-**8**.

***N*-(*all-trans*-Retinoyl)alanine Ethyl Ester (9).** The procedure for the preparation of **9** from L-alanine ethyl ester hydrochloride (20 mmol) and retinoic acid (10 mmol) was identical with the procedure for the preparation of L-**7**: yield, 3.5 g (87%, syrup); HPLC, 99.9% $[\alpha]^{25}_{589}$ +59.1° and $[\alpha]^{25}_{546}$ +82.1° (*c* 0.58 DMF).

***N*-(*all-trans*-Retinoyl)alanine (10).** The procedure for the preparation of L-**10** from L-**9** was identical with the procedure for the preparation of L-**8**: yield, 49% (pale yellow crystals from acetonitrile–water); mp 152–153 °C (inserted at 120 °C, shrinks at 135 °C); HPLC, 100%; $[\alpha]^{25}_{589}$ +76° and $[\alpha]^{25}_{546}$ +103° (c 1.05 in DMF); UV_{max} 347 nm (ε 51 300); ^1H NMR (300.64 MHz, $CDCl_3$) δ 1.03 (s, 16 + 17), 1.48 (m, 2 + 3′), 1.62 (m, 3), 1.72 (s, 18), 2.01 (s, 19), 2.04 (m, 4), 2.36 (s, 20), 4.63 (m, 2′), 5.72 (s, 14), 6.13 (m, 7 or 8, 10, NH), 6.24 (m, 12), 6.27 (m, 8 or 7), 6.96 (d of d, 11), 10.71 (br, COOH). Anal. ($C_{23}H_{33}NO_3$) C, H, N.

When L-**9** was treated with potassium hydroxide in absolute ethanol for 2.5 h and the reaction mixture was concentrated without neutralizing the excess base, the isolated product was predominantly racemic. The concentrated reaction mixture was diluted with water, the aqueous solution was acidified to pH 3.5, and the resulting mixture was extracted with ether (3×). The ether extract was washed with sodium chloride solution, dried ($MgSO_4$), and concentrated in vacuo to a yellow solid that was recrystallized from acetonitrile: mp 167–169 °C; HPLC, 100%; $[\alpha]^{23}_{589}$ +19.1° (*c* 1 in DMF). This material was used in the bioassays; the optical rotation compared to that of L-**10** above indicated that this specimen was about 77% DL-**10**.

***N*-(*all-trans*-Retinoyl)-DL-tyrosine (12).** *N*-(*all-trans*-Retinoyl)-DL-tyrosine ethyl ester (DL-**11**) was prepared by the procedure described for the preparation of (L-**7**); yield, 90% after column chromatography; UV_{max} 349 nm (ε 47 500).

The ethyl ester (DL-**11**) was hydrolyzed by the procedure described for the preparation of L-**8** except that the reaction time was 6 h. The product was recrystallized twice from acetonitrile–water: yield of DL-**12**, 46%; mp 172–173 °C (inserted at 160 °C); HPLC, 99.3%; UV_{max} 347 nm (ε 50 100), 226 (ε 13 100); ^1H NMR (100.1 MHz, Me_2SO-d_6) δ 1.01 (s, 16 + 17), 1.3–1.8 (m, 2, 3), 1.68 (s, 18), 1.95 (s, 19), 2.0 (m, 4), 2.22 (s, 20), 2.7–3.1 (m, 3′), 4.4 (m, 2′), 5 (br, OH), 5.9 (s, 14), 6.14–6.5 (m, 7, 8, 10, 12), 6.7–7.1 (m, 11), 6.66 (m, phenyl), 7.04 (m, phenyl), 8.16 (d, NH), 9.16 (br, COOH). Anal. ($C_{29}H_{37}NO_4$) C, H, N.

***N*-(*all-trans*-Retinoyl)-DL-glutamic Acid (14).** *N*-(*all-trans*-Retinoyl)glutamic acid diethyl ester (DL-**13**) was prepared by the procedure described for the preparation of L-**7**: yield, 68%; HPLC, 97.3%. The diethyl ester (DL-**13**, 6.8 mmol) was hydrolyzed (15.5 mmol of KOH) by the procedure described for the preparation of L-**8**. The product was recrystallized twice from acetonitrile–water: yield of DL-**14** (pale yellow crystals), 27%; mp 153–154 °C (inserted at 140 °C); HPLC, 99.9%; UV_{max} 348 nm (ε 50 000); ^1H NMR (300.64 MHz, Me_2SO-d_6) δ 1.02 (s, 16 + 17), 1.45 (m, 2), 1.57 (m, 3), 1.70 (s, 18), 1.80 (m, 3′), 1.97 (s, 19), 2.01 (m, 4), 2.28 (s, 20), 2.31 (m, 4′), 4.26 (m, 2′), 5.90 (s, 14), 6.16 (m, 7), 6.25 (m, 8), 6.27 (m, 10), 6.31 (m, 12), 6.93 (d of d, 11), 8.19 (d, NH), 12.37 (m, COOH). Anal. ($C_{25}H_{35}NO_5$) C, H, N.

***N*-(13-*cis*-Retinoyl)-L-leucine ethyl ester (15)** was prepared from L-leucine ethyl ester hydrochloride (20 mmol) and 13-*cis*-retinoic acid (10 mmol) by the procedure described for the preparation of L-**7**: yield of L-**15**, 91% (syrup); HPLC, 98.7%; $[\alpha]^{25}_{589}$ −110° and $[\alpha]^{25}_{546}$ −150° (c 0.48 in DMF).

***N*-(13-*cis*-Retinoyl)-L-leucine (16)** was prepared from L-**15** by the procedure described for the preparation of L-**8** except that the reaction time was 2.5 h. The initial precipitate (93% yield, $[\alpha]^{25}_{589}$ −156° (*c* 1.0 in DMF)) was recrystallized twice from acetonitrile–water: 32% yield of L-**16**; mp 134–135 °C (inserted at 125 °C); HPLC (80:20 acetonitrile–1% aqueous ammonium acetate), 99.8%; $[\alpha]^{25}_{589}$ −153° and $[\alpha]^{25}_{546}$ −203° (*c* 0.98 in DMF); UV_{max} 347 nm (ε 45 900); ^1H NMR (300.64 MHz, $CDCl_3$) δ 0.96 (m, 5′ + 6′), 1.03 (s, 16 + 17), 1.48 (m, 2), 1.61 (m, 3 + 1 H of 3′), 1.71 (s, 18), 1.74 (m, 4′ + 1 H of 3′), 1.98 (s, 19), 2.01 (m, 4), 2.03 (s, 20), 4.63 (m, 2′), 5.58 (s, 14), 6.04 (d, NH), 6.13 (m, 8 or 7), 6.23 (m, 10), 6.25 (m, 7 or 8), 6.91 (d of d, 11), 7.78 (d, 12), 9.58 (br, COOH). Anal. ($C_{26}H_{39}NO_3$) C, H, N.

***N*-(13-*cis*-Retinoyl)phenylalanine Methyl Ester (17).** Compound L-17 was prepared from L-phenylalanine methyl ester hyrochloride (20 mmol) and 13-*cis*-retinoic acid (10 mmol) by the procedure described for the preparation of L-7: yield of L-17, 72%; HPLC, 98.6%; $[\alpha]^{25}_{589}$ +29.1° and $[\alpha]^{25}_{546}$ +44.1° (c 1.23 in DMF).

Compound DL-17 was prepared in the same manner from DL-phenylalanine methyl ester hydrochloride (20 mmol) and 13-*cis*-retinoic acid (10 mmol): yield, 83%; HPLC, 98%; $[\alpha]^{25}_{589}$ 0° (DMF).

***N*-(13-*cis*-Retinoyl)phenylalanine (18).** The L-phenylalanine methyl ester (L-17) was hydrolyzed by the procedure described for the preparation of L-8. The initial precipitate (HPLC, 100%; $[\alpha]^{25}_{589}$ +55.9° and $[\alpha]^{25}_{546}$ +76.8° (c 1.06 in DMF)) was recrystallized from acetonitrile–water, and the yellow crystalline solid was triturated with acetonitrile: yield of L-18: 42%; mp 143–145 °C (inserted at 135 °C); $[\alpha]^{25}_{589}$ +58.7° and $[\alpha]^{25}_{546}$ +82.6° (c 1.02 in DMF). As expected, the solid-state IR spectrum of L-8 showed similarities to, but also differed from, the solid-state spectrum of the DL form (below).

The DL-phenylalanine methyl ester (DL-17) was hydrolyzed according to the procedure described for the preparation of L-8. The initial precipitate (HPLC, 100%) was recrystallized from acetonitrile–water: 58% of DL-18; mp 154–155 °C (inserted at 140 °C); UV_{max} 350 (ε 44 900); ^1H NMR (100.1 MHz, $CDCl_3$) δ 1.03 (s, 16 + 17), 1.3–1.8 (m, 2, 3), 1.71 (s, 18), 1.84–2.16 (m, 4), 1.98 (s, 19), 2.01 (s, 20), 3.0–3.4 (m, 3′), 4.92 (m, 2′), 5.51 (s, 14), 5.96 (m, NH), 6.1–6.5 (m, 7, 8, 10), 6.92 (m, 11), 7.1–7.5 (m, phenyl), 7.7 (d, 12), 10.3 (m, COOH). Anal. ($C_{29}H_{37}NO_3$) C, H, N.

Hydrolysis of L-17 (KOH in ethanol) at 60 °C and concentration of the alkaline solution to near dryness resulted in racemization; the yield of isolated DL-18 was 49%.

***N*-(13-*cis*-Retinoyl)alanine (20).** The ethyl ester (19) of *N*-(13-*cis*-retinoyl)alanine was prepared from L-alanine ethyl ester hydrochloride (20 mmol) and 13-*cis*-retinoic acid (10 mmol) according to the procedure described for the preparation of L-7: yield of L-19 (syrup), 80%; HPLC, 98%; $[\alpha]^{25}_{589}$ −196° and $[\alpha]^{25}_{546}$ −256° (c 0.82 in DMF).

The ethyl ester (L-19) was hydrolyzed according to the procedure described for the preparation of L-8 except that the reaction time was 2 h. The initial precipitate (94% yield, $[\alpha]^{25}_{589}$ −218° and $[\alpha]^{25}_{546}$ −287° (c 1.27 in DMF)) was recrystallized from acetonitrile–water: yield of yellow crystals (L-20), 57%; mp 139–142 °C (inserted at 130 °C); HPLC, 100%; $[\alpha]^{25}_{589}$ −243° and $[\alpha]^{25}_{546}$ −320° (c 1.08 in DMF); UV_{max} 350 nm (ε 44 900); ^1H NMR (100.1 MHz, $CDCl_3$) δ 1.02 (s, 16 + 17), 1.3–1.8 (m, 2, 3), 1.48 (d, 3′), 1.71 (s, 18), 1.84–2.2 (m, 4), 1.98 (s, 19), 2.04 (s, 20), 4.62 (m, 2′), 5.58 (s, 14), 6.0–6.2 (m, NH), 6.0–6.5 (m, 7, 8, 10), 6.96 (m, 11), 7.78 (d, 12), 10.5 (br, COOH). Anal. ($C_{23}H_{33}NO_3$) C, H, N.

When L-19 was hydrolyzed (KOH in ethanol) at 60 °C and the alkaline reaction mixture concentrated without neutralizing the excess base, considerable racemization occurred. The concentrated reaction mixture was diluted with water, the aqueous mixture was washed with ether, the aqueous layer was then acidified, and the resulting syrupy precipitate was extracted into ether. The residual syrup from the ether extract amounted to a quantitative yield of 19 and assayed 99% by HPLC. Crystallization of the crude product from acetonitrile and recrystallization of the yellow solid from the same solvent afforded 19 that was partially racemized: $[\alpha]^{23}_{589}$ −156° (c 1 in DMF); yield, 36%; mp 155–157 °C (inserted at 140 °C); HPLC, 99.9%. This material (about 36% DL-20) was used in the bioassays.

***N*-(13-*cis*-Retinoyl)glycine Ethyl Ester (21).** A dry solution of ethyl glycinate free base in ether was prepared, as described previously,[28] from ethyl glycinate hydrochloride (19.6 g, 140 mmol). A solution of 13-*cis*-retinoyl chloride[28] (2b) in benzene, prepared from 4.5 g (15 mmol) of 13-*cis*-RA, was added dropwise to the cold (10 °C) solution of ethyl glycinate, and the cold mixture was stirred for 1.5 h. Cold water (45 mL) was added to the reaction solution, the resulting mixture was concentrated in vacuo at 30 °C to remove the organic solvents, the aqueous mixture was extracted with ether (100 mL), a small amount of sodium chloride was added to facilitate the separation of layers, and the aqueous layer was extracted again with ether (50 mL). The ether extract (combined portions) was dried ($MgSO_4$) and concentrated in vacuo to an orange syrup that crystallized. The crude product (5 g) was triturated with cold ethanol,[35] and the crystalline product (3.3 g, 57% yield, mp 112–114 °C) was recrystallized from ethanol: yield, 2.8 g (48%); mp 115–116 °C (inserted at 100 °C); HPLC, 99.8%; UV_{max} 351 nm (ε 41 300), 242 (ε 8700); ^1H NMR (300.64 MHz, $CDCl_3$) δ 1.03 (s, C16, C17), 1.30 (t, OCH_2CH_3), 1.47 (m, C2), 1.62 (m, C3), 1.70 (s, C18), 1.99 (s, C19), 2.02 (m, C4), 2.04 (s, C20), 4.10 (d, C2′), 4.23 (q, OCH_2CH_3), 5.61 (s, C14), 6.04 (t, NH), 6.13 (m, C7 or C8), 6.22 (m, C7 or C8, C10), 6.91 (m, C11), 7.81 (d, 12).

***N*-(13-*cis*-Retinoyl)glycine (22)** was prepared from the ethyl ester 21 (11.1 mmol) and potassium hydroxide (15.5 mmol, 87% KOH) in 95% ethanol according to the procedure described for the preparation of 6 except that the reaction time was 1.5 h. The initial precipitate (98% yield, mp 85–88 °C) was recrystallized from ethanol: yield, 80%; mp 85–88 °C; HPLC, 99.6%; UV_{max} 348 nm (ε 47 700), 242 (9000); ^1H NMR (300.64 MHz, $CDCl_3$) δ 1.02 (s, C16, C17), 1.24 (t, CH_3 of ethanol), 1.45 (m, C2), 1.59 (m, C3), 1.69 (s, C18), 1.94 (s, C19), 1.96 (s, C20), 2.0 (m, C4), 3.22 (q, CH_2 of ethanol), 3.93 (s, C2′), 5.68 (s, C14), 6.10 (m, C7 or C8), 6.17 (m, C10), 6.20 (m, C8 or C7), 6.82 (m, C11), 7.25 (NH), 7.80 (d, C12), 6.0–7.0 (COOH + OH of ethanol). Anal. ($C_{22}H_{31}NO_3 \cdot C_2H_5OH \cdot H_2O$) C, H, N.

Hamster Tracheal Organ Culture Assay. The tracheal organ culture bioassay described by Sporn and co-workers[10-12] measures the ability of retinoids to reverse keratinization in retinoid-deficient tracheas. Tracheas were removed from 29–31-day-old hamsters that had been maintained on a vitamin A free diet and were placed in serum-free CMRL Medium 1066 containing bovine insulin, 1.0 μg/mL; hydrocortisone hemisuccinate, 0.1 μg/mL; glutamine, 2 mM; and Garamycin, 50 μg/mL. The cultures were placed in an amber plastic chamber, gassed with 50% oxygen–45% nitrogen–5% carbon dioxide and then rocked on a platform rocker in a 36 °C incubator. The tracheas were maintained in culture medium containing no retinoid for the first 3 days. At the end of 3 days, the tracheas were placed in fresh culture medium and divided into three groups as follows: the first group was treated with the retinoid dissolved in DMSO, final concentration of DMSO 0.1%; the second group was treated with *all-trans*-retinoic acid (reference substance) dissolved in DMSO; and the third group was treated with an equivalent amount of DMSO alone. All tracheas were maintained in culture for an additional 7 days; culture medium and gas atmosphere were changed three times during the 7-day treatment. At the end of 10 days (7 days after the first retinoid treatment), all tracheas were fixed in 10% buffered formalin. Each trachea was cut into two equal portions and embedded in paraffin. Eight cross-sections of 5 μm were made and stained with hematoxylin and eosin-phloxine B.

The histological status of the tracheal epithelium was assessed with respect to both the presence of keratin and keratohyaline granules and the extent of squamous metaplasia. Tracheas were scored as active if neither keratin nor keratohyaline granules were seen; they were scored as inactive if both keratin and keratohyaline granules were seen. Cultures were graded as to the percentage of their total epithelium showing squamous metaplasia: *none*; *minimal* if less than 2% of the epithelium was squamous; *mild* if between 2% and 10% was squamous; *marked* if between 10% and 40%; and *severe* if greater than 40%. Dose–response curves were made from log-transformed data by using a least-squares polynomial cubic regression model. The ED_{50}, which was the molarity of retinoid required to reverse keratinization in 50% of the cultures, was determined from these results.

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(35) The recovery was higher when the crude product was triturated with acetonitrile.

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Supplementary Material Available: Infrared spectral data and MS data for compounds 4, 6, 8, 10–12, 14, 16, 18, and 20–22 (5 pages). Ordering information is given on any current masthead page.

Triazolines. 14.[1] 1,2,3-Triazolines and Triazoles, a New Class of Anticonvulsants. Drug Design and Structure-Activity Relationships

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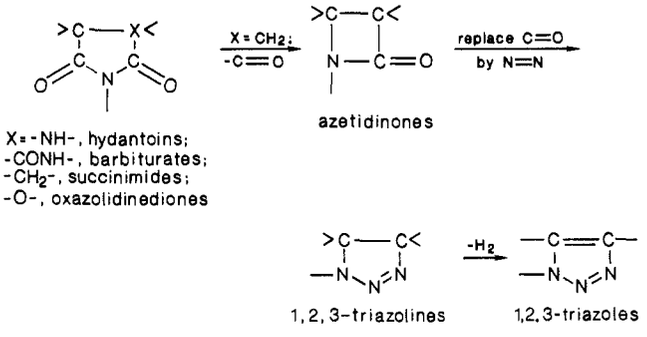
Pioneering studies in our laboratories have led to the emergence of the Δ^2-1,2,3-triazolines (4,5-dihydro-1*H*-1,2,3-triazoles) and the closely related 1*H*-1,2,3-triazoles as a unique family of anticonvulsant agents hitherto unknown. Unlike the traditional anticonvulsants, the dicarboximide moiety is absent from the triazoline ring system. This paper examines the results of evaluation of several groups of 1-aryl-5-pyridyl-substituted triazolines and triazoles with particular reference to structure–activity relationships in each compound group as well as between compounds in the different groups and the 1,5-diaryl compounds. The Topliss manual approach for application of the Hansch method is employed for the rational design of triazoline/triazole anticonvulsants. Anticonvulsant activity was determined, after intraperitoneal administration, in two standard seizure models in the mouse, the MES and scMet tests. Central nervous system toxicity was evaluated in the rotorod ataxia test. Analysis of structure–activity relationships using the Topliss scheme indicated a clear $\pi + \sigma$ dependency in the 1-aryl-5-(4-pyridyl)triazolines while an adverse steric effect (E_s) from 4-substitution appeared to be present in the 1-aryl-5-(3-pyridyl) compounds. A similar but strong steric effect dominated the structure–activity pattern of the 1-aryl-5-(4-pyridyl)triazoles, although a σ dependency was more evident in the 1-aryl-5-(3-pyridyl)- and the 1,5-diaryltriazole series. No significant activity was observed among the 1-aryl-5-(2-pyridyl)triazolines, and although the respective triazoles were active, the parameter dependency was not clearly defined. Similarly, the 1,5-diaryltriazolines, as a group, showed no pronounced anticonvulsant activity. However, replacement of the 5-aryl with a pyridyl group, particularly a 4-pyridyl, led to highly enhanced anticonvulsant activity. In addition, oxidation of triazolines with no anticonvulsant activity yielded, as a rule, triazoles that were active, which could be linked to their chemistry or structural conformation. The triazolines and triazoles evince anticonvulsant activity as a class and compare very well with the prototype antiepileptic drugs—ethosuximide, phenytoin, phenobarbital, valproate—in their anticonvulsant potency and minimal neurotoxicity. They have emerged as a new generation of anticonvulsant agents that show great promise as potentially useful antiepileptic drugs.

Pioneering studies in our laboratories have led to the emergence of the Δ^2-1,2,3-triazolines[2] (4,5-dihydro-1*H*-1,2,3-triazoles) and the closely related 1*H*-1,2,3-triazoles[3] as a unique family of anticonvulsant agents hitherto unknown.[4-6] In an earlier paper, screening results for a series of 1,5-diaryl-1,2,3-triazolines were reported.[7] This paper examines the results of evaluation of several groups of 1-aryl-5-pyridyl-substituted triazolines and triazoles with particular reference to structure–activity relationships in each compound group as well as between compounds in the different groups and the 1,5-diaryl compounds. The Topliss manual approach[8-10] for application of the Hansch method[11] is employed for the rational design of triazoline/triazole anticonvulsants.

Scheme I



The triazoline ring system is unique; it is different from those of the conventional anticonvulsant drugs, the majority of which have a dicarboximide (CONHCO) or a ureide (NHCONH) function, as in barbiturates, hydantoins, succinimides, and oxazolidinediones or the closely related primidones. The absence of the dicarboximide

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