7b (7.9 g, 22 mmol) in dry benzene (80 mL) under N_2 while maintaining the temperature below 25 °C. After 30 min, dimethylcarbamoyl chloride (2.9 g, 27 mmol) in benzene (5 mL) was added, and the reaction mixture was stirred at ambient temperature overnight. The mixture was cooled in ice and diluted with diethyl ether (100 mL) and water (25 mL). The organic layer was separated, washed with brine, dried (Na_2SO_4), and filtered, and the filtrate was evaporated to give 9.2 g (97%) of an oil containing a mixture of isomers **9a** and **9b** (ratio ca. 1:2). The product ratio was determined by integration of the NMR signal for the pyridine ring methyl resonances (δ 2.42 and 2.26) of **9a** and **9b**, respectively. This mixture was used without further purification.

***N,N*-Dimethyl-*N'*-[4-(hydroxymethyl)-6-methyl-2-pyridyl]-*N'*-[2-(diisopropylamino)ethyl]urea (10a) and *N,N*-dimethyl-*N'*-[6-(hydroxymethyl)-4-methyl-2-pyridyl]-*N'*-[2-(diisopropylamino)ethyl]urea (10b).** A solution of the mixture of isomeric benzyl ethers **9a** and **9b** (9.1 g, 21 mmol) in glacial acetic acid (75 mL) was hydrogenated over 10% Pd/C (3.0 g) under 50 psi of hydrogen in a Parr apparatus over a period of 3 days. The catalyst was removed by filtration, and the solvent was evaporated to give a residual oil, which was dissolved in methylene chloride. This solution was washed with dilute sodium hydroxide solution and then brine, dried (Na_2SO_4), and evaporated to give 5.3 g of a mixture of the debenzylated isomers. Chromatography of this mixture on silica gel eluting with chloroform saturated with ammonia gave 2.6 g (37%) of **10b** and 1.7 g (24%) of **10a** as oils. The NMR spectra were identical with that reported for the isolated metabolites.[3] Each isomer was dissolved in ethanol, treated with an equivalent of ethanolic HCl, and the respective solutions were diluted with diethyl ether to give the crystallin hydrochloride salts of **10b**, mp 168–170 °C, and **10a**, mp 210–212 °C. Anal. ($C_{18}H_{32}N_4O_2$·HCl) C, H, N.

2-[[2-(Diisopropylamino)ethyl]amino]-4,6-bis(trifluoromethyl)pyridine (8). A solution of **6** (16.2 g, 37.7 mmol) in 50% sulfuric acid (130 mL) was refluxed for 12 h. The cooled solution was diluted with water (200 mL) and neutralized with 10 N sodium hydroxide. The resultant oil was extracted into methylene chloride, dried (Na_2SO_4), and evaporated to give 12.5 g (93%) of **8** of high purity.

***N,N*-Dimethyl-*N'*-[4,6-bis(trifluoromethyl)-2-pyridyl]-*N'*-[2-(diisopropylamino)ethyl]urea (11).** A solution of 1.34 M *n*-butyllithium in hexane (29.6 mL, 40 mmol) was added to 2,2,6,6-tetramethylpiperdine (6.55 g, 46 mmol) in dry benzene (110 mL) under N_2. At 10-min intervals, a solution of **8** (11.8 g, 33 mmol) in benzene (30 mL) was added, followed by a solution of dimethylcarbamoyl chloride (4.5 g, 42 mmol) in benzene (30 mL). After stirring overnight at ambient temperature, the reaction mixture was quenched with methanol (6 mL) and saturated Na_2CO_3 (30 mL). The organic layer was separated, and the aqueous layer was extracted with methylene chloride. The combined organic extracts were washed with brine, dried (Na_2SO_4), and filtered and the filtrate was evaporated to give 15.0 g of crude product. This material was twice distilled to give 9.8 g (69%) of **11**, bp 122–124 °C (0.2 mm). Anal. ($C_{18}H_{26}F_6N_4O$) C, H, N.

***N,N*-Dimethyl-*N'*-[4,6-bis(trideuteriomethyl)-2-pyridyl]-*N'*-[2-(diisopropylamino)ethyl]urea (12).** A solution of **1** (10.6 g, 33 mmol) in dimethyl-d_6 sulfoxide (35 mL) containing potassium *tert*-butoxide (100 mg) was heated under nitrogen at 90 °C for 21 h. The reaction mixture was diluted with methylene chloride, thoroughly washed with water, dried (Na_2SO_4), and filtered through a charcoal pad, and the solvent was evaporated. The residue was dissolved in 2-propanol, and 1 equivalent of ethanolic HCl was added. Upon dilution with diethyl ether, **12** precipitated and, after recrystallization from 2-propanol, gave 9.5 g (79%) of **12** as the hydrochloride salt, mp 190–193 °C. Anal. ($C_{18}H_{26}D_6N_4O$·HCl) C, H, N.

The progress of this exchange reaction was monitored by the disappearance of the methyl resonances (4-CH_3, δ 2.22; 6-CH_3, δ 2.37) of **1** in the NMR. The 4-CH_3 resonance disappeared appreciably before any measurable exchange at the 6-CH_3 position occurred.

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Registry No. 1, 75308-65-5; **2**, 75329-62-3; **3**, 75329-65-6; **4**, 1522-22-1; **5a**, 75329-63-4; **5a** acid, 75329-66-7; **5b**, 75329-64-5; **5b** acid, 75329-67-8; **6**, 86569-00-8; **7a**, 75329-68-9; **7b**, 75329-69-0; **8**, 86569-01-9; **9a**, 75329-71-4; **9b**, 75329-70-3; **10a**, 75329-72-5; **10a**·HCl, 75329-74-7; **10b**, 75329-53-2; **10b**·HCl, 75329-73-6; **11**, 86569-02-0; **12**, 86569-03-1; **12**·HCl, 75338-52-2; *N,N*-diisopropyl-2,2-ethanediamine, 121-05-1; ethyl (ethoxycarbonyl)-acetimidate hydrochloride, 2318-25-4; dimethylcarbamoyl chloride, 79-44-7.

Synthesis and Pharmacological Activity of 6-[(*E*)-2-(2,6,6-Trimethyl-1-cyclohexen-1-yl)ethen-1-yl]- and 6-(1,2,3,4-Tetrahydro-1,1,4,4-tetramethyl-6-naphthyl)-2-naphthalenecarboxylic Acids

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6-[(*E*)-2-(2,6,6-Trimethyl-1-cyclohexen-1-yl)ethen-1-yl]- and 6-(1,2,3,4-tetrahydro-1,1,4,4-tetramethyl-6-naphthyl)-2-naphthalenecarboxylic acids (4 and 8) have been synthesized and show significant activity in reversing the keratinization process in hamster tracheal organ culture and in inhibiting the induction of ornithine decarboxylase in mouse skin by 12-*O*-tetradecanoylphorbol-13-acetate, two assays used to measure retinoid activity. The 2-naphthalenecarboxylic acid **8** was more active than **4**.

The potential value of retinoids as therapeutic agents for the treatment and chemoprevention of such diseases as epithelial cancer, psoriasis, and acne[1] has increased interest in retinoid chemistry and the study of retinoid structure–activity relationships and has led to the design of new retinoid analogues.[2-5] For these reasons, we report new structural modifications of the retinoid skeleton, 6-[(*E*)-2-(2,6,6-trimethyl-1-cyclohexen-1-yl)ethen-1-yl]- and

Scheme I

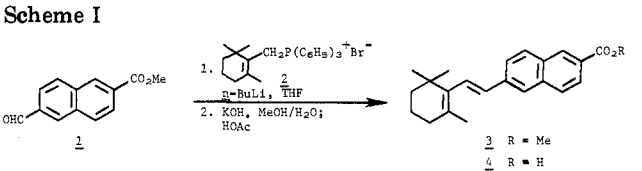

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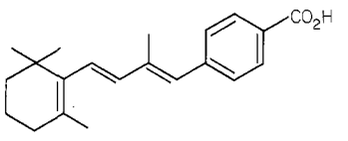
Table I. Biological Assay Results for Retinoids 4 and 8

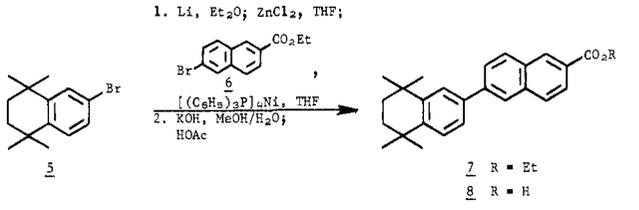
retinoid	reversal of keratinization in hamster tracheal organ culture[a] concn, M	active/total cultures, %	rel act.[c]	inhibn of induction of ODC by TPA in mouse skin[b] dose, nmol	% inhibn of control
4	10^{-8}	7/7 (100)	0.4	17.0	48
	10^{-9}	11/12 (92)		1.7	30
	10^{-10}	6/13 (46)			
	10^{-11}	1/5 (20)			
8	10^{-9}	7/7 (100)		17.0	80
	10^{-10}	21/22 (95)	>10	1.7	56
	10^{-11}	17/22 (77)			
	10^{-12}	4/15 (27)			
9			1.0[d]	17.0	77
				1.7	62

[a] Reference 3a. [b] Reference 6. ODC = ornithine decarboxylase. TPA = 12-*O*-tetradecanoylphorbol-13-acetate. [c] Relative activity is defined as the reciprocal of the ratio of the ED_{50} (retinoid)/ED_{50} (retinoic acid) divided by the ratio of the ED_{50} (9)/ED_{50} (retinoic acid), where the ED_{50} is the dose of retinoid required to reverse keratinization in 50% of the cultures. When 4 was tested, the ED_{50} for the retinoic acid control was 6×10^{-12} M; for 8 this control value was 7×10^{-12}, and for 9 it was 3×10^{-11}. [d] Reference 2b.

6-(1,2,3,4-tetrahydro-1,1,4,4-tetramethyl-6-naphthyl)-2-naphthalenecarboxylic acids (4 and 8). The bonds of 4 and 8 that correspond to the 9,11,13*E* double bond system of retinoic acid are restricted to a planar cisoid conformation by the naphthalene ring. Nevertheless, these analogues display significant activity in two bioassays that are used to assess retinoid activity, namely, (1) the reversal of keratinization of epithelial cells in hamster tracheal organ culture[3a] and (2) the inhibition of the induction of ornithine decarboxylase in mouse dorsal epidermis by a tumor-promoting phorbol ester.[6] In fact, **8** is the first non-olefinic compound reported that displays retinoid activity.

Chemistry. Naphthalenecarboxylic acid **4** was prepared in two steps from 6-carbomethoxy-2-naphthalenecarboxaldehyde (**1**) by the route shown in Scheme I. Reaction of **1** with β-cyclogeranylidenetriphenylphosphorane, which was prepared from the corresponding phosphonium salt (**2**) and *n*-BuLi, afforded ester **3** and its *Z* isomer in a ratio of 8:1 by LC analysis. Hydrolysis of **3** gave **4**. The trans geometry of the double bond of **3** and **4** was established by their ^1H NMR spectra that displayed two doublets having coupling constants of 16 Hz for the vinylic protons. In contrast, the ^1H NMR spectrum of the purified *Z* isomer of the ester had two vinylic proton doublets (δ 6.22 and 6.58) having a 12.6-Hz coupling constant.

Scheme II



The ethyl ester of naphthalenecarboxylic acid **8** was prepared from 6-bromo-1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (**5**)[7] and ethyl 6-bromo-2-naphthalenecarboxylate (**6**)[8] by the synthetic sequence shown in Scheme II. The unsymmetrical biaryl coupling was effected by using methodology developed by Negishi,[9] namely, reaction of the arylzinc reagent (obtained by treatment of bromotetrahydronaphthalene **5** with lithium, followed by metal exchange with $ZnCl_2$) and bromonaphthalene **6** in the presence of the catalyst tetrakis(triphenylphosphine)nickel(0). Hydrolysis of the ester afforded **8**.

Pharmacological Activity. Preliminary biological testing results are presented in Table I. The activity of **4** in the reversal of keratinization assay is at least an order of magnitude less than that of benzoic acid **9**, which we had synthesized previously. Analogue **4** is also less active in the ornithine decarboxylase assay. In **9**, the bonds corresponding to the 11,13*E* double bond system of retinoic acid are restricted to a cisoid conformation by the phenyl ring. Further restriction of the bonds corresponding to the 9,11*E* double bond system to a cisoid conformation may account for the reduced activity of **4**. Both **4** and **9** are less active than retinoic acid. However, some analogues of **9**

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show enhanced chemopreventive activity. For example, ethyl 4-[(*E*)-2-(1,2,3,4-tetrahydro-1,1,4,4-tetramethyl-6-naphthyl)-1-propen-1-yl]benzoate is more active in the antipapilloma assay than its analogue having an (*E*,*E*)-4-carbethoxy-3-methyl-1,3-butadien-1-yl group in place of the 4-carbethoxyphenyl ring.[4] Both are more active than retinoic acid and its ethyl ester. A similar enhancement of activity was found with the naphthalenecarboxylic acid analogue 8, which was more active than 4 in both bioassays. In fact, 8 was more active than retinoic acid in the reversal of keratinization assay. However, in the ornithine decarboxylase assay, 8 showed 64 and 93% of the activity of retinoic acid at the 1.7- and 17-nmol dose levels, respectively. The difference in activities may be due to differences in the two bioassay systems. Compounds such as the (tetrahydronaphthyl)naphthalenecarboxylic acid 8 are more readily synthetically accessible and far more stable than retinoic acid and may have potential as therapeutic agents.

Experimental Section

Where required, reactions and purifications were conducted with deoxygenated solvents and under inert gas (argon) and subdued light. Solvents were dried or distilled before use. TLC analyses were performed on Analtech silica gel analytical plates. Merck silica gel 60 was used for chromatography. Melting points are uncorrected. LC analyses were done on a Waters Associates ALC 210 equipped with a Radialpak A or B cartridge. Detection was by a Schoeffel Instrument Model 770 variable-wavelength UV monitor. Analyses were performed at ambient temperature. IR spectra were recorded with a Perkin-Elmer 710B infrared spectrophotometer. NMR spectra were obtained with a Varian EM360A or XL-100-F or a 300-MHz Nicolet spectrometer, with Me_4Si as an internal standard (δ 0) and solvent as specified. UV spectra were taken on a Perkin-Elmer 552 spectrophotometer. High-resolution mass spectral analyses were conducted on a CEC-21-110B high-resolution mass spectrometer equipped with facilities for combination GC–MS.

Methyl 6-[(*E*)-2-(2,6,6-Trimethyl-1-cyclohexen-1-yl)ethen-1-yl]-2-naphthalenecarboxylate (3). To a suspension of 1.39 g (2.9 mmol) of β-cyclogeranyltriphenylphosphonium bromide (2) in 40 mL of THF at −20 °C was added 1.88 mL (2.9 mmol) of a 1.54 M solution of *n*-BuLi in hexane. The mixture was stirred for 20 min at 0 °C, at which time almost all of the phosphonium salt went into solution. To the dark brown solution was added 0.62 g (2.9 mmol) of 6-carbomethoxy-2-naphthalenecarboxaldehyde (1) in 5 mL of THF. The reaction mixture became light yellow. It was stirred at room temperature for 24 h and then diluted with 200 mL of H_2O and extracted with Et_2O (3 × 100 mL). The Et_2O layer was dried (Na_2SO_4), concentrated, and filtered through 100 g of silica gel to give 0.8 g (82% yield) of a very pale yellow oil: LC (Radialpak B, 1% Et_2O/hexane, 2 mL/min, 260 nm) t_R 3.8 (12%, *Z* isomer), 4.1 min (88%, **3**); IR (film) 2950, 1730, 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.12 [s, 6, C-$(CH_3)_2$], 1.3–1.8 [2 m, 4, $(CH_2)_2$], 1.82 (s, 3, C=CCH_3), 2.05 (m, 2, C=CCH_2), 3.97 (s, 3, CO_2CH_3), 6.52 (d, J = 16 Hz, ~1, HC=C*H*Ar), 6.95 (d, J = 16 Hz, ~1, *H*C=CHAr), 7.7–8.7 (m, 6, ArH); MS calcd for $C_{23}H_{26}O_2$, 334.1933; found, 334.1930.

6-[(*E*)-2-(2,6,6-Trimethyl-1-cyclohexen-1-yl)ethen-1-yl]-2-naphthalenecarboxylic Acid (4). A suspension of 0.75 g (2.25 mmol) of **3** in 5 mL of MeOH was degassed twice under argon before a solution of 1.0 g (17.8 mmol) of KOH in 2 mL of H_2O and 3 mL of MeOH was added. The reaction mixture was degassed twice more and heated under reflux for 30 min. It was cooled and acidified with about 15 mL of 50% H_2O/HOAc. The product was extracted with Et_2O (2 × 50 mL). The Et_2O extracts were washed with brine, dried (Na_2SO_4), and evaporated to give a light yellow solid, which was recrystallized from EtOAc/hexane to give 0.54 g (75% yield) of **4** as pale yellow crystals: mp 173–174 °C; LC (Radialpak A, 50% H_2O/MeCN, 2 mL/min, 260 nm) t_R 1.4 min (100%); IR (mull) 2800–3200 (broad), 1700, 1620 cm^{-1}; 300-MHz ^{1}H NMR ($CDCl_3$/Me_2SO-d_6) δ 1.11 [s, 6, $C(CH_3)_2$], 1.55 and 1.65 [2 m, 4, $(CH_2)_2$], 1.81 (s, 3, C=CCH_3), 2.05 (m, 2, C=CCH_2), 6.54 (d, J = 16 Hz, 1, HC=C*H*Ar), 6.90 (d, J = 16 Hz, 1, *H*C=CHAr), 7.73 (d, J = 8.5 Hz, 1, 4-ArH), 7.77 (s, 1, 5-ArH), 7.86 and 7.93 (2 d, J = 8.5 Hz, 2, 7,8-ArH), 8.10 (d, J = 8.5 Hz, 1, 3-ArH), 8.67 (s, 1, 1-ArH); ^{13}C NMR ($CDCl_3$/Me_2SO-d_6) 18.7, 21.4, 28.7, 32.5, 33.8, 39.1, 124.2, 124.9, 128.9, 129.4, 129.6, 130.1, 131.4, 131.9, 135.3, 137.0, 137.3, 167.3 ppm; UV (EtOH) λ_{max} 254 nm (ϵ 2.71 × 10^4), 317 (2.41 × 10^4); MS calcd for $C_{22}H_{24}O_2$, 320.1776; found, 320.1786.

Ethyl 6-(1,2,3,4-Tetrahydro-1,1,4,4-tetramethyl-6-naphthyl)-2-naphthalenecarboxylate (7). The coupling procedure of Negishi[9] was adapted. A solution of 4.138 g (15.5 mmol) of 6-bromo-1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene[7] in 8 mL of Et_2O was added under argon to a stirred mixture of 0.60 g (0.086 mol) of Li wire (1% Na) cut in small pieces, which had been prewashed with MeI and Et_2O, in 7 mL of Et_2O at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, at which time an aliquot treated with water/hexane showed no aryl bromide by GC analysis (0.125 in. × 6 ft, 3% OV-1, 120 °C, 2 min, 16 °C/min to 250 °C). After cooling to −10 °C, the supernatant solution of aryllithium reagent was added to a stirred solution of 2.15 g (15.6 mmol) of fused $ZnCl_2$ in 24 mL of THF, which had been precooled to −5 °C. After 0.5 h at 0 °C, the resultant arylzinc mixture was added to a stirred solution of 3.67 g (13.2 mmol) of ethyl 6-bromo-2-naphthalenecarboxylate[8] and 0.28 g (0.25 mmol) of tetrakis(triphenylphosphine)nickel(0) in 24 mL of THF at 5 °C. The dark reaction mixture was allowed to warm slowly to 20 °C over a 30-min period and maintained there for 30 min. It was then poured into 100 g of ice and 30 mL of cold 10% HCl and extracted with Et_2O (150 mL, 2 × 50 mL). Washing with saturated $NaHCO_3$ and brine, drying ($MgSO_4$), and concentrating at reduced pressure afforded a crystalline residue, which was dissolved in 250 mL of Et_2O. The solvent was concentrated to about 15 mL, diluted with 15 mL of hexane, and cooled to −80 °C to give 3.47 g of white crystals. Impurities were removed by chromatography on 90 g of silica gel with benzene to afford 3.3 g of material. Crystallization from Et_2O at −20 °C gave 2.42 g (48% yield) of **7** as shiny, colorless plates: mp 155.5–156.5 °C; TLC (10% acetone/hexane) R_f 0.68; LC (Radialpak B, 1% EtOAc/hexane, 2 mL/min, 260 nm) t_R 4.3 min (100%); LC (Radialpak A, MeCN, 2 mL/min, 280 nm) t_R 5.4 min (100%); IR (mull) 1630, 1365, 1280, 1255, 920, 890, 815, 770, 750 cm^{-1}; 300-MHz ^{1}H NMR ($CDCl_3$) δ 1.34 and 1.38 [2 s, 12, $C(CH_3)_2$], 1.45 (t, J = 7.1 Hz, 3, CH_2CH_3), 1.74 [s, 4, $(CH_2)_2$], 4.45 (q, J = 7.1 Hz, 2, CH_2CH_3), 7.43 (d, J = 8.6 Hz, 1, 8-ArH), 7.50 (dd, J = 1.9 and 8.6 Hz, 1, 7-ArH), 7.66 (d, J = 1.9 Hz, 1, 5-ArH), 7.80 (dd, J = 1.7 and 8.7 Hz, 1, 7-naphthyl H), 7.92 (d, J = 8.6 Hz, 1, 4-naphthyl H), 8.01 (d, J = 8.7 Hz, 1, 8-naphthyl H), 8.03 (br s, 1, 5-naphthyl H), 8.09 (dd, J = 1.7 and 8.5 Hz, 1, 3-naphthyl H), 8.62 (br s, 1, 1-naphthyl H); ^{13}C NMR ($CDCl_3$) 14.3, 31.8, 31.9, 34.1, 34.4, 35.0, 35.1, 60.9, 124.7, 125.1, 125.5, 126.4, 127.1, 127.4, 128.1, 129.6, 130.6, 131.4, 135.7, 137.6, 141.1, 144.6, 145.4, 166.6 ppm; UV (EtOH) λ_{max} 231 nm (ϵ 4.4 × 10^4), 264 (4.6 × 10^4), 312 (2.3 × 10^4); MS calcd for $C_{27}H_{30}O_2$ 386.2246; found, 386.2221.

6-(1,2,3,4-Tetrahydro-1,1,4,4-tetramethyl-6-naphthyl)-2-naphthalenecarboxylic Acid (8). A suspension of 0.434 g (1.12 mmol) of **7** in a solution of 0.628 g (9.5 mmol) of 85% KOH in 1.1 mL of water and 10 mL of EtOH was heated at reflux for 10 min, at which time solution was achieved and TLC (40% acetone/hexane) showed no starting material (R_f 0.90). The reaction mixture was diluted with 20 mL of cold water and acidified with HOAc. The precipitated product was extracted into Et_2O (2 × 100 mL). The extracts were washed with brine, dried ($MgSO_4$), and concentrated at reduced pressure to give 0.396 g of white crystalline product. Recrystallization from 45 mL of acetone at −20 °C afforded 0.309 g (77% yield) of acid as colorless crystals: mp 287.5–288.5 °C; LC (Radialpak A, 60% MeOH/water, 2 mL/min, 260 nm) t_R 15.1 min (100%); IR (mull) 1680, 1630, 1300, 1220, 910, 880, 810, 770, 740 cm^{-1}; 300-MHz ^{1}H NMR (Me_2SO-d_6) δ 1.29 and 1.35 [2 s, 12, $C(CH_3)_2$], 1.69 [s, 4, $(CH_2)_2$], 3.40 (br s, 1, CO_2H), 7.46 (d, J = 8.3 Hz, 1, 8-ArH), 7.57 (dd, J = 2.0 and 8.3 Hz, 1, 7-ArH), 7.75 (d, J = 2.0 Hz, 1, 5-ArH), 7.92 (dd, J = 1.6 and 8.7 Hz, 1, 4-naphthyl H), 8.00 (dd, J = 1.6 and 8.7 Hz, 1, 7-naphthyl H), 8.09 (d, J = 8.7 Hz, 1, 8-naphthyl H), 8.19 (d, J = 8.8 Hz, 1, 3-naphthyl H), 8.26 (br s, 1, 5-naphthyl H), 8.63 (br s, 1, 1-naphthyl H); ^{13}C NMR (Me_2SO-d_6) 31.5, 33.7, 34.0, 34.6, 124.3, 124.6, 124.9, 125.4, 125.9, 127.0, 127.8, 128.3, 129.7, 130.1, 131.1, 135.2, 136.7, 140.1, 144.2, 144.9, 167.4 ppm; UV (EtOH)

λ_{max} 227 nm (ϵ 4.1 × 10^4), 260 (5.5 × 10^4), 307 (2.1 × 10^4); MS calcd for $C_{25}H_{26}O_2$, 358.1933; found, 358.1938.

Biological Assays. The experimental protocols used for the reversal of keratinization assay and the inhibition of the induction of ornithine decarboxylase assay were essentially those described by the groups of Sporn[3a] and Verma and Boutwell,[6] respectively.

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Registry No. 1, 7567-87-5; **2**, 56013-01-5; **3** (*E* isomer), 86471-12-7; **3** (*Z* isomer), 86471-17-2; **4** (*E* isomer), 86471-13-8; **5**, 27452-17-1; **6**, 86471-14-9; **7**, 86471-15-0; **8**, 86471-16-1; ornithine decarboxylase, 9024-60-6.

An Analysis of 1-(2-Chloroethyl)-1-nitrosourea Activity at the Cellular Level†

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The effect of five different 1-(2-chloroethyl)-1-nitrosoureas on the growth of cultured P388 cells has been analyzed in terms of physical, chemical, and kinetic parameters that are related to the mechanism of action of this class of cancer chemotherapeutic agent. This study correlates structure with activity at the cellular level by using a dose function that is related to the amount of active species, the (2-chloroethyl)diazonium ion, that is formed during the period of exposure of cells to drug rather than to the initial drug dose. 1-(2-Chloroethyl)-1-nitrosourea analogues that rapidly enter the P388 cells are shown to have the same activity relative to the amount of active species formed. When analyzed in this way, activity is not influenced by the structure of the N-3 substituent, lipophilicity, or carbamoylating activity. The agents 1-(2-chloroethyl)-1-nitrosourea (CNU), 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea (PCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) all produce a 50% cell growth inhibition at 6 to 7 μM active species formed per cell volume. Chlorozotocin required a twofold higher effective dose to produce the same toxic effect. This decreased activity is attributed to the slow uptake of the water-soluble chlorozotocin into P388 and L1210 cells relative to the rate of chlorozotocin conversion to active species in medium. The yields to 2-chloroethanol from CNU, BCNU, and chlorozotocin were shown to be the same, indicating that these agents generate the same yield of alkylating intermediate at 37 °C and pH 7.4.

The 1-(2-chloroethyl)-1-nitrosoureas are a class of chemotherapeutic agents with demonstrated clinical activity against a variety of malignant diseases,[1-3] particularly brain tumors[4,5] and lymphomas.[6] The mechanism of action of these agents involves the chemical conversion of the parent nitrosourea to active alkylating species, the (2-chloroethyl)diazonium ion, and substituted isocyanates.[7-9]

Quantitative structure–activity relationships have been developed for a large number of 3-substituted 1-(2-chloroethyl)-1-nitrosoureas by using intraperitoneally and intracerebrally inoculated L1210 mouse leukemia[10-12] and Lewis lung carcinoma.[13] These studies show that in vivo activity correlates with only one physical parameter, lipophilicity, and structural indicators. A further examination of 17 active nitrosoureas suggested that carbamoylating activity, as well as lipophilicity, contributed to the toxicity in L1210 leukemic mice. Alkylating activity was thought to be a major factor in determining the quantity of compound required to give a therapeutic response.[14] Subsequent studies demonstrated a significant correlation between in vivo toxicity and alkylating activity; no correlation was found with carbamoylating activity, however. Neither parameter showed any correlation with antitumor activity.[15] The ultimate biological effect of the 1-(2-chloroethyl)-1-nitrosoureas is now thought to result from covalent binding of alkylating and carbamoylating species in cellular macromolecules. There is much evidence that the antitumor effect is due to alkylation and cross-linking of DNA by (2-chloroethyl)diazonium ion.[16-20] The

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