Retinylnitrones: A New Class of Retinoids with Chemopreventive Action

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Abstract: Retinoids containing a polar nitrone end group have been synthesized. This new class of extensively conjugated, open chain nitrones has been found to be effective in reversing the keratinization in hamster tracheal organ cultures. The highest activity was displayed by the retinylnitrone bearing the least bulky N-alkyl substituent, i. e., methylnitrone **1**; its activity level was comparable to that of all-*trans* retinoic acid.

Normal growth, reproduction, vision and epithelial cell differentiation require the presence of vitamin A, deficiency of which leads to pathological changes of epithelial tissues. The absence of vitamin A or its derivatives in organs and tissues maintained in culture, e. g., hamster trachea, mouse prostate and epidermis, chick embryo, etc., causes preneoplastic lesions in them. Vitamin A derivatives and compounds having structures modelled after it, "the retinoids", have been shown to be effective in chemoprevention of cancer (1). Retinoids prevent or reduce neoplasia caused by vitamin A deficiency or induced by carcinogens in epithelial tissues of animals. The role of retinoids in controlling epithelial cell differentiation in various organ cultures has also been demonstrated by *in vitro* experiments. In the light of recent strides made in the use of these compounds in the chemoprevention of cancer, it is imperative to develop new and improved retinoids with enhanced pharmacokinetic properties and reduced toxicity. The present communication reports the synthesis and properties of a new class of compounds, retinyl nitrones, and their ability *in vitro* to control epithelial cell differentiation.

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Results and Discussion

In order to obtain retinoids with improved pharmacological properties, synthetic modifications of the trimethylcyclohexenyl ring, the polyene side chain, or the end group of vitamin A molecule can be envisaged. Among these possibilities, the alteration of the end group by a polar nitrone function was attractive. Although there has been no report on extensively conjugated open chain nitrones, the pentaene system of retinal is expected to exert a stabilizing effect on the polar structure of these nitrones. The nitrone group, an important intermediate in the synthesis of natural products (2), is also known to confer a variety of biological activities, e. g., it is a pharmacophore present in therapeutic agents such as the tranquilizer chlordiazepoxide (3). Recently it has also been reported that α-phenyl-N-alkyl nitrones can act as neoplasm inhibitors (4).

The nitrones **1–8** (Fig. 1) could be prepared readily from all-*trans* retinal (or its 13-*cis* isomer in case of **9–10**) and the appropriate N-alkylhydroxylamine in moderate to high yields by following a general procedure for the preparation of aldonitrones (5). Thus, nitrone **1** was obtained by treatment of an etheral solution of all-*trans* retinal with N-methylhydroxylamine hydrochloride in aqueous $NaHCO_3$ for 24 hours. Upon evaporation of the ether layer, orange crystals were obtained which after recrystallizations from ether/hexane (m. p. 139–141°, yield of 95 %) were found to be pure by high performance liquid chromatography (HPLC, on Partisil PXS 10/25 ODS-2 column from Whatman, eluted with 2 % water in methanol). Chemical ionization mass spectra confirmed the molecular weight (M^++1 at 314) and showed a loss of 16 mass unit, typical for nitrones (6). The chemical shifts in the ^{1}H-NMR (250 MHz, $CDCl_3$) δ at 7.37 (d, J=10 Hz, 15-H), 6.80 (dd, J=14, 10 Hz, 11-H), 6.71 (d, J=10 Hz, 14-H), 6.39 (d, J=14 Hz, 12-H), 6.22 (d, J=14 Hz, 7-H), 6.13 (d, J=10 Hz, 10-H), 6.11 (d, J=14 Hz, 8-H), 3.72 (s, N-CH_3), 1.97 and 1.96 (s, 13- and 9-CH_3), 1.69 (s, 5-CH_3), 1.01 (s. 1,1'-CH_3) were in agreement with the structure (*Z*)-α-[(*all-E*)-2,6-dimethyl-8-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3,5,7-octatetraenyl]-N-methylnitrone, which was further confirmed by decoupling experiments and ^{13}C-NMR shifts. Thus a single isomeric form (*Z*) of methylnitrone **1** was obtained, and the (*E*)-isomer could not be detected. In the case of *tert*-butylnitrone **2**, the (*E*)-isomer was the more stable and the sole product. In the other cases, NMR analysis showed the presence of both (*Z*) and (*E*)-isomers at room temperature; however, amounts sufficient for testing each of a pair of geometric isomers could be obtained only with the cyclohexyl derivatives **5** and **6**.

Nitrones vary considerably in their stability: acyclic nitrones are generally readily hydrolyzed by acids to the corresponding carbonyl derivatives, and some alkyl substituted nitrones are even decomposed by hydroxylic solvents, whereas certain N-*tert*-butyl derivatives are known to resist acid hydrolysis (7). Retinylnitrones were stable in hydroxylic solvents and dimethyl sulfoxide and were remarkably stable under acidic conditions. For example, nitrone **1** showed no decomposition in aqueous methanol, nor was it hydrolyzed by 10 % aqueous HCl/methanol (1:1). Treatment of **1** with 10 % aqueous HCl in ethanol/hexane for 10 minutes at room temperature gave nitrone salt **11**.

Reaction of **1** with the dipolarophile phenylisocyanate in CH_2Cl_2 for 65 hours at room temperature led to the stable oxadiazolidinone **12**; UV(MeOH) 330 nm, IR (film) 1730 cm^{-1}, DCI/MS 433 (M^++1), 389 (M^+-CO_2), and ^{1}H-NMR ($CDCl_3$) δ at 2.92 (N-CH_3), 5.44 (d, br, 15-H), 7.14–7.28 (aromatics). Nitrone **1** is stable in the dark at room temperature in the presence of air, but exposure to roomlight in methanol solution for 10 minutes converts it into a 40/

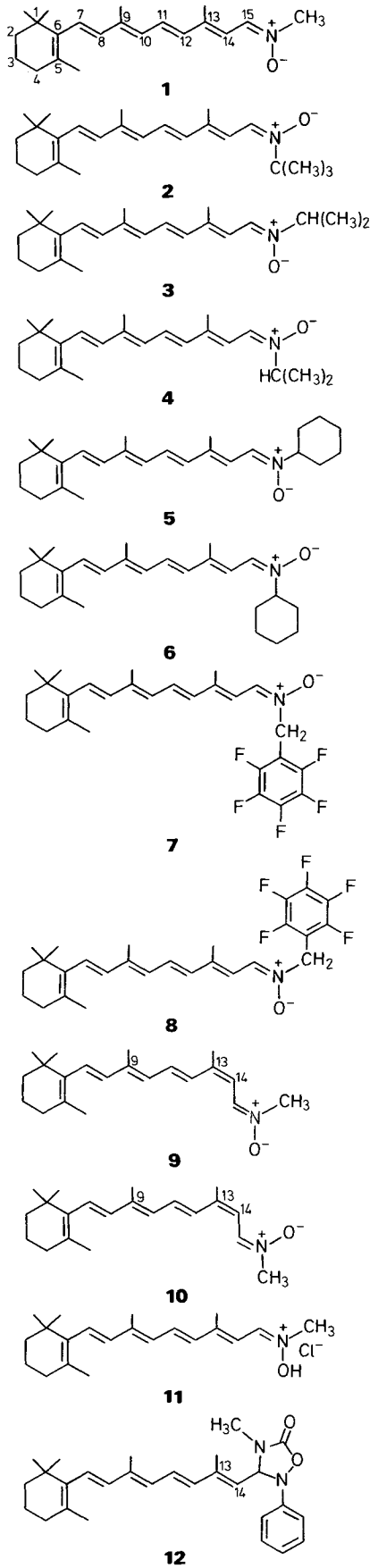
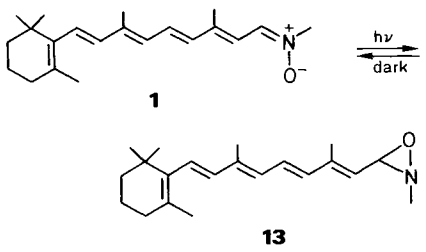


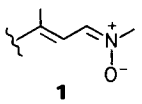
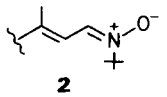
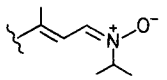
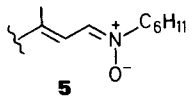
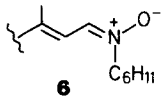
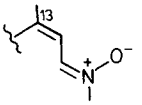
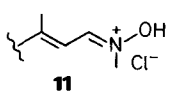
Fig. 1 Structure of retinylnitrones and derivatives.

60 mixture with the corresponding oxaziridine **13**. The oxaziridine can be separated from the mixture by HPLC and can be partially reconverted to the nitrone by keeping it in the dark.



Nitrones **1–10** all show red-shifted absorption maxima at ca. 440 nm in aqueous HCl/MeOH, which are reversibly blue-shifted to ~ 400 nm upon addition of base. All nitrones **1-10** are light sensitive. Unlike compound **1**, the other nitrones and derivatives could not be obtained in pure form by recrystallizations alone; they were therefore separated by thin layer chromatography on silica gel and finally purified for testing by HPLC (Partisil M9 10/50 ODS-2 column, eluted with 3 to 10% water in methanol). The structure in each case was ascertained by combined spectral

Table I. Physical Properties and Activity of Retinylnitrones.

Compound	M.p. (°C)	UV (MeOH)	Activity[a]
Retinoic acid[b]			Active 419/474 cultures at 10^{-9}M Active 134/256 cultures at 10^{-10}M
 1	139–141	400 (ε=64,000)	Active 7/8 cultures at 10^{-9}M Active 3/7 cultures at 10^{-10}M
 2	114–115	402 (ε=57,700)	Active 10/16 cultures at 10^{-9}M Active 9/17 cultures at 10^{-10}M
 4	153–154	400 (ε=63,000)	Inactive 4/7 cultures at 10^{-9}M Inactive 6/7 cultures at 10^{-10}M
 5	139–140	400 (ε=67,200)	Inactive 3/5 cultures at 10^{-9}M Inactive 5/6 cultures at 10^{-10}M
 6	52–53	394 (ε=53,900)	Inactive 4/7 cultures at 10^{-9}M Inactive 4/6 cultures at 10^{-10}M
 10	oil	390 (ε=80,200)	Active 7/7 cultures at 10^{-9}M Inactive 7/7 cultures at 10^{-10}M
 11	156–158	397 (ε=60,700) 387 (ε=58,100)	Active 7/7 cultures at 10^{-9}M Inactive 5/6 cultures at 10^{-10}M

(a) Determined by the reversal of keratinization in hamster tracheal organ culture assay: Sporn, M. B. Dunlop, N. M., Newton, D. L., Henderson, W. R. (1976) Nature (London) 263, 110–113, and references cited therein.
(b) Data for retinoic acid is shown for comparison. Its activity was tested in over 1000 trachea cultures obtained from hamsters raised on vitamin A deficient diet. See in Newton, D. L., Hederson, W. R., Sporn, M. B. in "Structure Activity Relationship of retinoids", National Cancer Institute Publication, October 11, 1978.

data (UV, IR, MS, NMR). Stereochemistry of isomeric nitrones (e.g. **5** and **6**) was established by comparing the chemical shifts of 15-H (deshielded in **6**, 7.64 ppm, as compared to **5**, 7.43 ppm) (7) as well as by decoupling and nuclear Overhauser effect experiments.

Among the new compounds tested in the tracheal organ culture assay (Table I), the N-methyl derivatives **1**, **10** and **11**) possess the highest activity; compound **1** having activity comparable to that of retinoic acid. Interestingly, compound **10** which has 13-*cis* geometry exhibited the same activity as the all-*trans* isomer **1** at 10^{-9} M; however, **10** was inactive at 10^{-10} M concentration. One cannot attribute this to the geometry of the 13 double bond alone since the nitrone salt of the all-*trans* isomer, compound **11**, displayed the same trend.

The effect on rabbit platelet aggregation of nitrone **1** was tested *in vitro* and it showed inhibitory activity against platelet aggregation induced by ADP or collagen (IC_{50}=38 and 55 μg/ml, respectively). Compound **1** also potentiates the submaximal platelet aggregation induced by a low concentration (0.1 mM) of arachidonic acid. In antibiotic assays, methylnitrone **1** showed weak to moderate anti-Staphilococcal and antifungal activity *in vitro*; however, tests in mice with lethal Staphilococcal infections failed to demonstrate significant systemic antibacterial activity. These preliminary results warrant further studies on retinylnitrones to determine their pharmacological action and structure activity relationship.

Acknowledgements

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