Bioorganic & Medicinal Chemistry Letters, Vol.2, No.9, pp. 1133-1136, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

4-OXYGENATED RETINOIDS: UNEXPECTED CHEMOPREVENTIVE POTENTIAL FOR ANALOGUES ORIGINALLY SYNTHESIZED AS AFFINITY LABELS

Robert W. Curley, Jr.* and Dean L. Carson

Division of Medicinal Chemistry and Pharmacognosy,
College of Pharmacy, The Ohio State University,
Columbus, Ohio 43210, USA

(*Received* 29 *May* 1992)

Abstract: Potential retinoid binding protein affinity labels **3** - **5** were prepared from the retinoic acid metabolite **2**. While unsuccessful as affinity labels, **5** and the serendipitously discovered ether **6** show potential as skin cancer chemopreventives, activity not normally associated with 4-oxygenated retinoids.

Retinoic acid (**1**) and its analogues (retinoids) are of much interest as agents useful for the treatment of skin disorders and as cancer chemopreventive agents.[1] The actions of these molecules are mediated by association with nuclear receptors and a number of other proteins are involved in the transport of these water insoluble ligands.[2] To aid in understanding the role of these proteins in the mechanism of action of **1** we have prepared a number of affinity matrices for the purification of these proteins using analogues of **1**.[3]

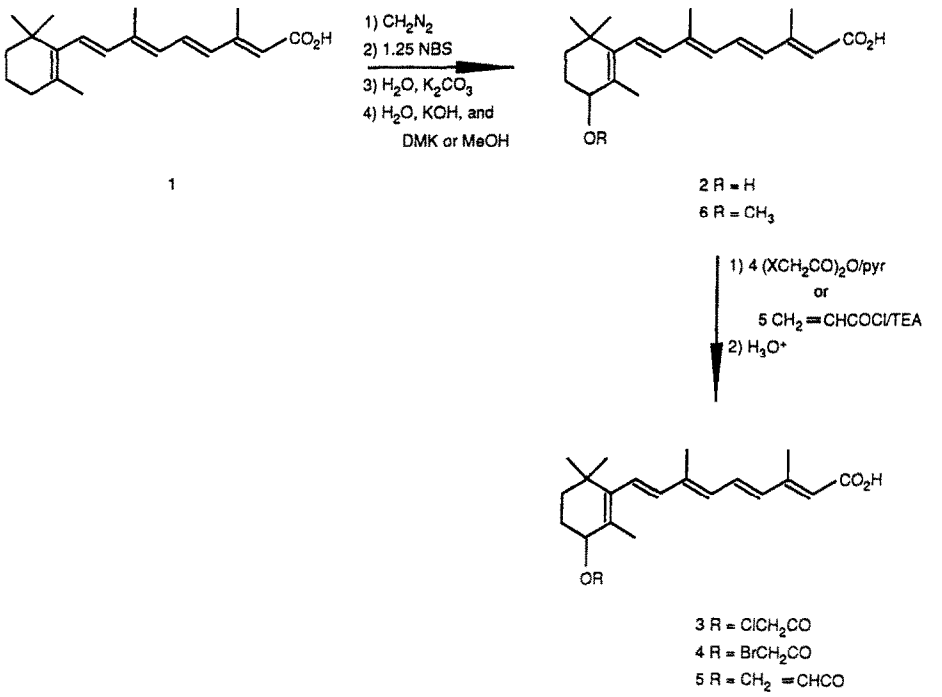
The acid **1** is fairly rapidly metabolized, principally to inactivation metabolites which are the result of 4-position oxygenation.[4] Despite the biological inactivity of the metabolite 4-hydroxyretinoic acid (**2**), this material does bind tightly to the cellular retinoic acid-binding protein (CRABP).[5] We have found that retinoids derived from **2** can be used to prepare useful ligands for affinity purification of retinoid-binding proteins[3] and have attempted to extend this work to the preparation of CRABP affinity labels **3**-**5**. While these materials have shown little utility as affinity labels for the CRABP, **5** and the serendipitously discovered ether **6** have shown unexpected potential as skin cancer chemopreventives.

Racemic metabolite **2** was prepared by modification of our previously published method.[6] Thus, allylic bromination of methyl retinoate followed by aqueous solvolysis and saponification yields **2** in 42% overall yield (Scheme I). Interestingly, when saponification is performed in aqueous methanolic KOH, the major product is the unexpected ether **6**. Confirmation of this structure was obtained by treating the allylic bromide derived from methyl retinoate with methoxide/methanol to give the methyl ester of **6** in 72% overall yield.

Haloacetylation of **2** with four equivalents of the appropriate anhydride gave 4-chloroacetoxy- and 4-bromoacetoxyretinoic acid (**3** and **4**) in 81% and 86% yields respectively after hydrolysis of the mixed anhydride of the carboxylate. Likewise, treatment of **2** with five equivalents of acryloyl chloride gave **5** as an 88:12 *trans*/13-*cis* isomer mixture in 88% yield.[7]

To assess the potential electrophilicity of the putative affinity labels, their relative reactivity with the model nucleophile 4-(4-nitrobenzyl)pyridine (NBP) was

determined. The procedure for the production of the chromogenic pyridinium salt from



electrophiles **3**-**5** was adapted from a previously published assay.[8] Results of this assay (Figure 1) showed acrylate **5** to be unreactive while haloacetates **3** and **4** alkylated NBP rapidly in the expected reactivity order. While **5** was thus not expected to be a useful affinity label, chloroacetate **3** was used to nonspecifically label human serum albumin (9:1 3/HSA found in conjugate). The resulting conjugate was stable to porcine hepatic carboxylesterase under conditions which rapidly cleaved retinyl acetate, however metabolite **2** was liberated by saponification. Unfortunately, neither **3** or **4** were found to be specific affinity labels for the target CRABP (J.M. Chapman and R.W. Curley, Jr., unpublished results). This lack of success is in contrast to earlier successes with 4-substituted retinoids as photoaffinity labels for retinoid-binding proteins[9] as well as other successes using haloacetates as affinity labels for proteins binding hydrophobic ligands.[10]

However, because of the apparent lack of electrophilicity of **5** and the possibility of useful pharmaceutical properties for **6**, these compounds were evaluated in an assay predictive for skin cancer chemopreventive potential. The ability of retinoids to inhibit tumor promoter-induced ornithine decarboxylase activity in mouse skin has long been known to correlate with inhibition of skin tumor promotion[11] and the assay has been used to distinguish active and inactive retinoids.[12] The activity of **1**, **5**, and **6** in this regard is shown in Table I.[13] From this data it appears the **5** has good activity comparable to **1** while **6** is only somewhat less effective. This surprising activity for derivatives of metabolite

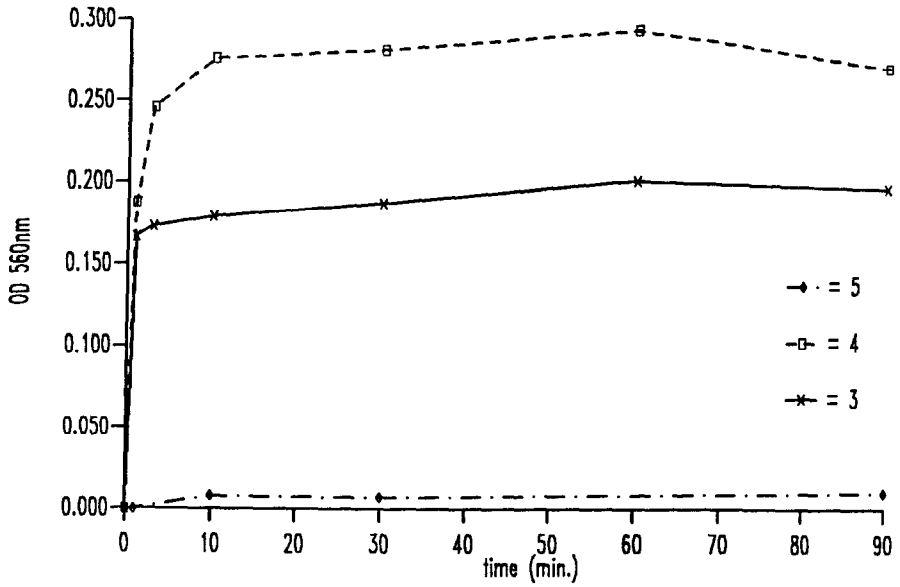


FIGURE 1. Model Nucleophile/Electrophilic Retinoid Reactions

2 suggests some favorable potential for 4-oxygenated retinoids in the dermatology area.

Table I. Retinoid Bioassay

compound	nmoles applied	ODC activity (nmoles CO_2/30 min/mg protein)
acetone (control)		0.17
TPA	17	10.0
1	1.7	2.0
1	17	1.9
4	1.7	2.3
4	17	2.5
5	1.7	3.5
5	17	2.4

Acknowledgements. We gratefully acknowledge the financial support of the National Institutes of Health (CA 40967) and the assistance of The Ohio State University Campus Chemical Instrument Center.

References and Notes

1. Moon, R.C.; Itri, L.M. in *The Retinoids*; Sporn, M.B.; Roberts, A.B.; Goodman, D.S., Eds.; Academic Press: Orlando, 1984, Vol. II, Chapter 14.

2. DeLuca, L.M. *FASEB J.* **1991**, *5*, 2924.

3. a) Chapman, J.M.; Curley, R.W., Jr. *Biochem. Biophys. Meth.* **1989**, *19*, 2871. b) Chapman, J.M.; Curley, R.W., Jr. *Protein Expression Purif.* **1990**, *1*, 63.

4. Newton, D.L.; Henderson, W.R.; Sporn, M.B. *Cancer Res.* **1980**, *40*, 3413.

5. Trown, P.W.; Palleroni, A.V.; Bohoslawec, O.; Richelo, B.N.; Halpern, J.M.; Gizzi, N.; Geiger, R.; Lewinski, C.; Machlin, L.J.; Jetten, M.E.R. *Cancer Res.* **1980**, *40*, 212.

6. Curley, R.W., Jr.; Carson, D.L. *Drug Des. Delivery* **1987**, *1*, 219.

7. For **3**: mp 114-116°C; UV (CH_2Cl_2) λ_{max} 360 nm (ε 44,500); ^1H NMR ($CDCl_3$) resembles **2**[6] with δ 4.08 (s,2, $ClCH_2COOCH$), 5.33 (t,1, $ClCH_2COOCH$); RP-HPLC (CH_3CN/50 mM NH_4OAc/THF 80:19:1) t_R = 6.5 min (97.6% of peak area); after treatment with CH_2N_2: MS, *m/e* (relative intensity) (M-$ClCH_2COOH$) 312 (89.1); Anal. Calcd for $C_{22}H_{29}O_4Cl$: Cl 9.02. Found 8.44. For **4**: mp 108-110°C; UV (CH_2Cl_2) λ_{max} 355 nm (ε 49,500); ^1H NMR ($CDCl_3$) resembles **2**[6] with δ 3.85 (s,2, $BrCH_2COOCH$), 5.30 (5,1, $BrCH_2COOCH$); RP-HPLC (CH_3CN/50 mM NH_4OAc/THF 80:19:1) t_R = 7.2 min (98% of peak area); after treatment with CH_2N_2: MS, *m/e* (relative intensity) (M-$BrCH_2COOH$) 312 (42.1); Anal. Calcd for $C_{22}H_{29}O_4Br$: Br 18.31. Found 18.80. For **5**: mp 112-116°C; UV (CH_2Cl_2) λ_{max} 362 nm (ε 39,500); ^1H NMR ($CDCl_3$) resembles **2**[6] with δ 5.30 (t,1, $CH_2CHCOOCH$), and 3 new vinyl protons in the δ 5.7 - 6.5 area; RP-HPLC (CH_3CN/50 mM NH_4OAc/THF 80:19:1) t_R = 4.2 and 7.5 min (98% of peak area assigned as 13-*cis* and all-*trans* isomers); after treatment with CH_2N_2: MS, *m/e* (relative intensity) (M^+) 384 (2.5), 312 (100). For **6**: mp 181-183°C; UV (CH_2Cl_2) λ_{max} 358 nm (ε 34,000); ^1H NMR ($CDCl_3$) resembles **1**[14] with δ 3.38 (s,3, CH_3OCH), 3.5 (t,1, CH_3OCH); HRMS, *m/e* required for $C_{21}H_{30}O_3$ 330.2196, observed 330.2222.

8. a) Katzenellenbogen, J.A.; McGorrin, R.J.; Tatee, T.; Kempton, R.J.; Carlson, K.E.; Kinder, D.H. *J. Med. Chem.* **1981**, *24*, 435. (b) A 50 nM solution of **3**,**4** or **5** was added to a 50 mM solution of NBP in 2:1 ethylene glycol/25 mM phosphate - 75 mM NaCl pH 7.2. At regular intervals 3 mL of solution was removed, mixed with 1:1 triethylamine/acetone, and the OD_{560} recorded vs. control without **3** - **5**.

9. a) Sheves, M.; Makover, A.; Edelstein, S. *Biochem. Biophys. Res. Commun.* **1984**, *122*, 577. b) Martin, C.A.; Dawson, M.I.; McCormick, A.M.; Napoli, J.L. *Biochem. Biophys. Res.Commun.* **1986**, *135*, 124.

10. a) Gawinowicz, M.A.; Goodman, D.S. *Biochemistry* **1982**, *21*, 1899; b) Chang, C.H.; Lobl, T.J.; Rowley, D.R.; Tindall, D.J. *Biochemistry* **1984**, *23*, 2527.

11. Verma, A.K.; Boutwell, R.K. *Cancer Res.* **1977**, *37*, 2196.

12. a) Dawson, M.I.; Hobbs, P.D.; Chan, R.L.; Chao, W.-R.; Fung, V.A. *J. Med. Chem.* **1981**, *24*, 583; b) Dawson, M.I.; Hobbs, P.D.; Chan, R.L.-S.; Chao, W.-R. *Ibid.* **1981**, *24*, 1214.

13. Test retinoids were applied to backs of shaved mice 30 minutes prior to application of the tumor promotor 12-O-tetradecanoylphorbol-13-acetate (TPA). Five hours later epidermal extracts were prepared and the specific activity of ornithine decarboxylase determined by measuring the release of $^{14}CO_2$ from ^{14}C-ornithine by enzymatic decarboxylation. Assays were performed at the Southern Research Institute. Unfortunately, we no longer have this relationship with SRI and have not yet been able to expand the quality of these interesting preliminary results.

14. Curley, R.W., Jr., Fowble, J.W. *Magn. Reson. Chem.* **1989**, *27*, 707.