Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 2, pp. 349-350, 1994
Copyright © 1994 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0960-894X/94 $6.00+0.00

0960-894X(93)E0086-G

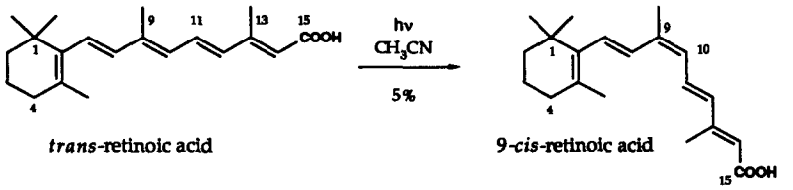
CONVENIENT PREPARATION OF 9-*CIS*-RETINOIC ACID

Jotham W. Coe* and Thomas O'Connell
Central Research Division, Pfizer Inc., Groton, CT. 06340

Abstract: 9-*cis*-Retinoic Acid was prepared in 5% yield by photoisomerization of all-*trans*-retinoic acid and simple recrystallization of the resulting isomeric mixture.

Retinoids have an important role in the control of cell differentiation and vertebrate development. Their activity is believed to be mediated through two classes of retinoid receptors:[1,2] retinoic acid receptors (RAR) and the recently identified retinoid "X" receptors (RXR).[3,4,5] The RXRs appear to have a bifunctional role: they serve as ligand-dependent transcription factors and as heterodimerization partners for other members of the nuclear receptor superfamily, including the vitamin D receptor, thyroid hormone receptor and RAR. The role of RXR in transcriptional regulation has attracted considerable interest, particularly since the identification of an endogenous ligand of RXR, 9-*cis*-retinoic acid (9-*cis*-RA).[6,7,8] Quantities of 9-*cis*-RA sufficient to support studies of RXR and its role in the regulation of gene transcription are therefore desirable, and disclosed herein is a convenient method for its preparation.

Historically 9-*cis*-RA has been obtained fortuitously as a result of non-selective olefination reactions in retinoic acid syntheses. The 9,10-*Z*-olefin has been formed as the minor isomer from Reformatskii[9,10] and Wittig[11] condensation reactions. Typically, these mixtures have been carried through subsequent operations to provide mixtures of isomeric retinoic acids. Purification in all reports has exploited the selective crystallization of 9-*cis*-RA or synthetic intermediates from mixtures.[9-12] The present method again exploits crystallization to enrich 9-*cis*-RA from a mixture of isomers, however total synthesis is not required: commercially available *trans*-retinoic acid serves as the starting material.



trans-retinoic acid — 9-*cis*-retinoic acid

Accordingly, *trans*-retinoic acid is photoisomerized in acetonitrile using a tungsten filament lamp until equilibrium is achieved (this contains ~14% 9-*cis*-RA, 24-36 h).[7] Concentration to half volume and standing at ambient temperature results in crystallization of a mixture of retinoic acids enriched in 9-*cis*-RA.[13] Attempts to alter the isomeric equilibrium or to achieve this selective enrichment by crystallization in other solvents have been unsuccessful (ether, ethanol). Filtration and a single recrystallization from ethanol provides 9-*cis*-RA, whose HPLC retention times and melting point are identical to published values.[7,10] Recycling of the mother liquors through photolytic re-equilibration allows significant quantities of the material to be prepared. Each cycle produces ~5% yield of 9-*cis*-RA.

Experimental:

Trans-retinoic acid (4.0 g, 13.3 mmol, Fluka) was dissolved in HPLC-grade acetonitrile (1.5 L) in a 3-liter single neck Pyrex flask equipped with a condenser, a nitrogen inlet tube and a magnetic stirrer. The stirred suspension dissolved at reflux under exposure to a 300 Watt tungsten filament lamp for 24 h (equilibration monitored by HPLC[7] or ^{1}H NMR[14]). After cooling, the solution was concentrated on a rotary evaporator (bath temperature 35-40 °C, aspirator vacuum) until 800 mL of solvent had been collected. On standing at ambient temperature for ~30 min crystallization began and was allowed to proceed for 10 minutes. Filtration provided 564 mg of a yellow solid.[15] Recrystallization from ethanol provided 197 mg of 9-*cis*-retinoic acid (mp 187-189 °C, lit. mp 189-191 °C;[10] 97.8%, HPLC,[14] 5% yield). Re-equilibration of the mother liquors under the above conditions provided 177 mg of additional material.[16]

References and Notes:

1. Gudas, L. J. *Cell Growth and Differentiation* **1992**, *3*, 655.
2. Leid, M.; Kastner, P.; Chambon, P. *Trends Biochem. Sci.* **1992**, *17*, 427.
3. Hamada, K.; Gleason, S. L.; Levi, B-Z.; Hirschfeld, S.; Appella, E.; Ozato, K. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 8289.
4. Mangelsdorf, D. J.; Ong, E.S.; Dyck, J. A.; Evans, R. M. *Nature* **1990**, *345*, 224.
5. Yu, V. C.; Delsert, C.; Andersen, B.; Holloway, J. M.; Devary, O. V.; Naar, A. M.; Kim, S. Y.; Boutin, J-M.; Glass, C. K.; Rosenfeld, M. G. *Cell* **1991**, *67*, 1251.
6. Levin, A. A.; Sturzenbecker, L. J.; Kazmer, S.; Bosakowski, T.; Huselton, C.; Allenby, G.; Speck, J.; Kratzeisen, C.; Rosenberger, M.; Lovey, A.; Grippo, J. F. *Nature* **1992**, *355*, 359.
7. Heyman, R. A.; Mangelsdorf, D. J.; Dyck, J. A.; Stein, R. B.; Eichele, G.; Evans, R. M.; Thaller, C. *Cell* **1992**, *68*, 397. Recently, a similar equilibrium mixture was reported in ethanol in a synthesis of radiolabelled 9-*cis*-RA. Purification was achieved by HPLC. See Dawson, M. I.; Hobbs, P. D.; Cameron, J. F.; Rhee, S. W. *J. Labelled Compd. Radiopharm.* **1993**, *33*, 245.
8. For a recent study involving 9-*cis*-retinoic acid and RXR, see Jong, L.; Lehmann, J. M.; Hobbs, P. D.; Harlev, E.; Huffman, J. C.; Pfahl, M.; Dawson, M. L. *J. Med. Chem.* **1993**, *36*, 2605.
9. For early work generating the 9-*cis*-olefin, see (a) Graham, W.; van Dorp, D. A.; Arens, J. F. *Rec. trav. chim. Pays-Bas.* **1949**, *68*, 609, also see, (b) van Dorp, D. A.; Arens, J. F. *Rec. trav. chim. Pays-Bas.* **1946**, *65*, 338, (c) Arens, J. F.; van Dorp, D. A. *Nature* **1946**, *157*, 190.
10. Robeson, C. D.; Cawley, J. D.; Weisler, L.; Stern, M. H.; Eddinger, C. C.; Chechak, A. J. *J. Am. Chem. Soc.* **1955**, *77*, 4111.
11. Pommer, H.; Sarnecki, W., Pat. No. DE 1050763, **1959**; *Chem. Abs.* **1961**, 5572f, *55*.
12. Garbers, C. F. *J. Chem. Soc.* **1956**, 3234.
13. a) Crystallization may be induced by seeding the solution, however this is not necessary. b) The mixture usually contains 55-75% 9-*cis*-RA, 25-38% *trans*-RA and < 7% other isomers.
14. (a) Registry No. 5300-03-8. (b) For HPLC conditions see ref. 7. (c) 9-*cis*-RA ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (dd, J = 11.5, 15.0 Hz, 1H, H_{11}), 6.66 (d, J = 16.0 Hz, 1H, H_8), 6.30 (d, J = 16.0 Hz, 1H, H_7), 6.26 (d, J = 15.0 Hz, 1H, H_{12}), 6.07 (d, J = 11.5 Hz, 1H, H_{10}), 5.81 (s, 1H, H_{14}), 2.37 (s, 3H, CH_3, C_{20}), 2.08 (m, 2H, CH_2, C_4), 2.02 (s, 3H, CH_3, C_{19}), 1.78 (s, 3H, CH_3, C_{18}), 1.66 (m, 2H, CH_2, C_3), 1.50 (m, 2H, CH_2, C_2), 1.07 (s, 6H, CH_3, $C_{16,17}$). Diagnostic signal indicating the 9-*cis*-isomer in an isomeric mixture, δ 6.66 (d, J = 16.0 Hz, 1H, H_8).
15. This mixture contains ~63% of the theoretical 9-*cis*-RA present at equilibrium.
16. (a) As a crystalline material the product is stored under nitrogen at 0 °C with no detectable isomerization or decomposition after 6 months. (b) The rate of equilibration has been observed to vary as a function of concentration, light intensity and light placement. (c) Overexposure to light or air leads to decomposition of the mother liquors.

(*Received in USA* 1 *October* 1993; *accepted* 2 *November* 1993)