

DEHYDRORETINOIC ACID FROM DEHYDRORETINAL

R. K. BARUA AND P. G. NAYAR

Department of Chemistry, University of Gauhati, Gauhati-14, Assam, India

INTEREST in retinoic acid has been renewed after Dowling and Wald¹ discovered that it has a high potency for growth but is ineffective in supporting the visual cycle. Further Thompson, Howell and Pitt² demonstrated that it is equally ineffective in reproduction. Ganguly³ has reviewed this aspect recently.

Dehydroretinoic acid—the congener acid of the dehydroretinal series—may be of interest, although in a limited field, as dehydroretinal cycles are hitherto found to be preponderant in the freshwater fishes only.

Dehydroretinoic acid was first obtained by Farrar, Henbest and Jones⁴ as an intermediate in the synthesis of dehydroretinal by bromination of synthetic methyl retinoate with N-bromosuccinimide and dehydrobromination of the resulting bromo compound. Recently, Isler and co-workers⁵ have prepared several isomers of dehydroretinoic acid by direct synthesis.

While the aldehydes of retinol and dehydroretinal can be readily obtained by oxidation of the corresponding alcohols by manganese dioxide following the method of Ball, Goodwin and Morton⁶ or its elegant modification by Wald,⁷ earlier attempts to prepare the acids directly by Henbest, Jones and Owen,⁸ were not successful. These authors could prepare retinoic acid by preparing the oxime of retinal, its dehydration to the nitrile by POCl_3 in pyridine and subsequent hydrolysis. Even then the reaction was slow under mild conditions and under drastic conditions lead to extensive decomposition. Recently Barua and Barua⁹ succeeded in the smooth oxidation of retinal to retinoic acid by use of Tollen's reagent (ammoniacal silver nitrate solution).

The less widely distributed dehydroretinol is more unstable than its congener retinol and it was thought worthwhile to see whether dehydroretinoic acid could be produced from the corresponding aldehyde dehydroretinal by use of the Tollen's reagent.

We report below a method for preparing dehydroretinoic acid starting from easily available freshwater fish liver oils.

Dehydroretinyl ester fraction (712 mg., $E_{1\text{ cm.}}^{1\%}$ at $350\text{ m}\mu = 655$ in light petroleum)

obtained by chromatography of *B. bagarius* liver oil was taken in ethanol (60 ml.) and an aqueous solution of potassium hydroxide (3 ml., 50% solution) was added. The mixture was heated on a water-bath (65°C.) for 20 min. under nitrogen. It was then cooled to room temperature and after dilution with water (70 ml.) was extracted with peroxide-free ether (50 ml.). The extraction was repeated twice with 50 ml. portions of ether. The combined ether extract was dried with anhydrous sodium sulphate, the ether removed in vacuum and the residue dissolved in light petroleum and chromatographed on deactivated alumina (100 g., 10% water). Dehydroretinol was eluted with light petroleum containing 8–10% ether. After a second chromatography with light petroleum solvent, the yield of dehydroretinol was 340 mg., $E_{1\text{ cm.}}^{1\%}$ at $350\text{ m}\mu = 1251$ in light petroleum.

A wad of cotton was introduced into the constriction of a glass chromatographic column (dia. 1.5 cm.) and manganese dioxide (8 g., B.D.H. precipitated) was packed into it. A solution of dehydroretinol, in light petroleum (10 ml.) was poured on the manganese dioxide column. Gentle pressure was applied to quicken the filtration. The filtrate was of a deep-orange colour characteristic of dehydroretinal. When the dehydroretinol solution had filtered down completely, the column was washed first with light petroleum (20 ml.) and then with a mixture of 2% ether in light petroleum (10 ml.). The combined filtrate was evaporated to dryness under reduced pressure and the residue dissolved in light petroleum and chromatographed on alumina (50 g., 8% water). Dehydroretinal which formed a bright orange-red zone on the column, was eluted with light petroleum containing 2% ether. It was chromatographed on deactivated alumina from light petroleum. Yield of dehydroretinal = 275 mg., $E_{1\text{ cm.}}^{1\%}$ at $385\text{ m}\mu = 1392$ in light petroleum.

50 ml. each, of 10% aqueous solutions of silver nitrate and sodium hydroxide were mixed in a 500 ml. conical flask and dilute ammonia was added until the solution was clear. A solution of dehydroretinal (0.050 g.)

in ethanol (10 ml.) was added followed by pure ethanol (100 ml.). The reaction mixture was then left in the dark at 25° C. for 1.5 hr. with occasional shaking. At the end of this period the solution was filtered to remove the black precipitate of silver. The filtrate was washed with ether to remove non-acidic impurities. It was then cooled in ice and 5 N hydrochloric acid was added until the solution was distinctly acid and precipitation of silver chloride complete. Crushed ice was added to the flask to check rise in temperature during neutralization. The solution was decanted into a separating funnel. The silver chloride residue was washed twice with distilled water and twice with dilute sodium hydroxide solution and all the washings were added to the funnel. After ensuring that the solution was acid it was extracted with light petroleum (50 ml.). The extraction was repeated with another 50 ml. of light petroleum. The combined extract was dried with anhydrous sodium sulphate, concentrated *in vacuo* and chromatographed on deactivated alumina (30 g., 8% water). On development with light petroleum, an orange band separated and flowed out of the column. This substance showed an ultra-violet absorption spectrum with flat maxima at 370 m μ and 330 m μ and was rejected. Dehydroretinoic acid remained strongly adsorbed on the column as a yellow zone. The column was washed with light petroleum containing 10% ether. It was then extruded and the dehydroretinoic acid zone was eluted with ethanol containing ammonia. The eluate was diluted with water and, after acidification with 5 N hydrochloric acid, extracted with light petroleum (40 ml.). The extract was dried with anhydrous sodium sulphate and concentrated under reduced pressure.

The residue was dissolved in a small quantity of light petroleum (B.P. 40–60°) and kept for 1 hr. in an ice-bath when dehydroretinoic acid separated as plates. The recrystallized material has the following characteristics:

$E_{1\text{ cm.}}^{1\%}$ at 370 m μ = 1337; at 304 m μ (shoulder) = 426 (solvent—light petroleum, ethanol), M.P. 180° (uncorr.). The colour produced by SbCl₃ reagent (recorded in a Beckman DK-2 spectrophotometer) gave $\lambda_{\text{max.}}$ at 643 m μ , $E_{1\text{ cm.}}^{1\%}$

643 m μ being 1213. On addition of a drop of 0.1(N) NaOH solution to the ethanolic solution of dehydroretinoic acid, the absorption maximum shifts to 360 m μ which could be brought back to 370 m μ on adding two drops of 0.1 N HCl (personal communication from Prof. R. A. Morton). The i.r. spectrum (nujol mull) of the acid showed acid carbonyl band at 1695 cm.⁻¹. The methyl ester of the acid was prepared by refluxing the acid with methyl iodide in ethyl acetate and potassium carbonate for 3 hr. at 50° C. The esterified product, was chromatographed on 8% weakened alumina, when the methyl ester was obtained which could be crystallized from methanol at -20°, as pale yellow needle-shaped crystals M.P. 43–45° (uncorr.), $\lambda_{\text{max.}}$ at 375 m μ , 307 m μ (inflexion) $E_{1\text{ cm.}}^{1\%}$ 375 m μ = 1322. When this methyl ester is reduced with lithium aluminium hydride dehydroretinol is obtained, $\lambda_{\text{max.}}$ 351, 287 m μ inflexion at 276 m μ , $E_{1\text{ cm.}}^{1\%}$ at 351 m μ being 1435 (solvent—ethanol). SbCl₃ colour $\lambda_{\text{max.}}$ at 693 m μ , $E_{1\text{ cm.}}^{1\%}$ being 3917. When this dehydroretinol is 'cyclised' with dry 0.03 N ethanolic HCl, the characteristic absorption bands of ethoxy anhydrovitamin A₂ at 350, 368 and 390 m μ were obtained. All these confirm the product as dehydroretinoic acid. The yield of dehydroretinoic from dehydroretinal is about 80% and is optimum when the reaction mixture in the oxidation experiment contained over 65% of ethanol.

1. Wald, G. and Dowling, J. E., *Proc. Nat. Acad. Sci., Wash.*, 1960, **46**, 587.
2. Thompson, J. N., Howell, J. Mc. C. and Pitt, C. A. J., *Biochem. J.*, 1961, **80**, 16.
3. Ganguly, J., *J. Sci. and Ind. Res.*, 1967, **26**, 1107.
4. Farrar, K. R., Hamlet, J. C., Henbest, H. B. and Jones, E. R. H., *Jour. Chem. Soc.*, 1952, p. 2657.
5. Isler *et al.*, *Helv. Chim. Acta*, 1962, **45**, 548.
6. Ball, S., Goodwin, T. W. and Morton, R. A., *Biochem. J.*, 1948, **42**, 516.
7. Wald, G., *J. Gen. Physiol.*, 1947, **31**, 489.
8. Henbest, H. B., Jones, E. R. H. and Owen, T. C., *J. Chem. Soc.*, 1952, p. 2657.
9. Barua, R. K. and Barua, A. B., *Biochem. J.*, 1962, **92**, 21 C.