An approach for conversion of retinoic acid to retinyl retinoate using dehydroretinol

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Retinoic acid is highly effective against photo aging. But its carboxyl end group results in a number of side effects. To overcome this difficulty an attempt has been made to develop a derivative of retinoic acid without carboxyl group using 3,4-didehydroretinol. 3,4-Didehydroretinol is purified from a natural source of Wallago attu fish liver. Both retinoic acid and didehydroretinol are allowed to react in presence of N,N-carbonyl diimidazole and dimethyl amino pyridine. The yield of the purified product retinyl retinoate is 55% with respect to didehydroretinol. The purified product is characterised with the help of UV-visible spectrophotometer, HPLC, LC-MS and NMR spectra. It is a new hybrid compound containing both retinoic acid and didehydroretinol.

Keywords: Retinoic acid, 3,4-didehydroretinol, retinyl retinoate, HPLC, LC-MS

Retinoids have been widely used for a long time for treatment of several skin diseases. The use of high dose retinoic acid as anti-acne drug can be explained on the basis of anti-proliferative effect of retinoic acid on human sebocytes. The intensity of protection depends on the intracellular concentration of retinoic acid. Several retinoids like retinol, retinyl ester, 3,4-dihydro retinoids, all trans-retinoic acid along with β-carotene have been identified in the human skin. Dehydroretinoic acid is equally as active as RA in terms of induction of parakeratosis in the reconstructed skin model. Retinoic acid receptor and retinoid X-receptor can mediate the biological response of skin to retinoic acid application. So synthetic retinoic acid or its derivative can be applied topically to overcome skin problems. In a recent study it has been shown that retinoic acid reduces the migratory capacity of neuro blastoma cells and invasiveness. But use of retinoic acid creates some difficulties like skin burning and hypervitaminosis A syndrome. Fat solubility of retinoic acid and the side effects have resulted in restricted pharmaceutical use of the compound as such. Many new derivatives of retinoids like retinyl β-glucuronide (ROG), retinoyl β-glucuronide (RAG), retinyl retinoate (RR), 6-OH-11-O-hydroxyphenanthrene (IIF) have already been synthesized to overcome these difficulties. Didehydroretinol has 1/3rd biological activity as compared to that of retinol. On investigation of normal human epidermis it is found that 20-25% of the total retinoid content is dehydroretinol. It is not reduced by irradiation as the is retinol reduced. Fresh water small fish like mola (Amblypharyngodon mola), which are the source of both vitamin A and dehydroretinol, are also found to be very effective for the children in Bangladesh. So didehydroretinol has been selected as the substrate with one hydroxyl end group to combine with carboxyl end group of retinoic acid. Herein is reported the synthesis of the new derivative of retinoid having 3,4-didehydroretinol as one of the components.

Experimental Section

The fish liver samples have been collected from the local fish market Maligaon (Assam, India) and are first dried with blotting paper as far as possible. The weighed partially dried sample is cut into small pieces and ground in a mortar with anhydrous NaSO_4 and extracted with hexane. Finally the hexane extracts are dried over anhydrous NaSO_4. The filtered extracts are collected in a round bottomed flask and concentrated on a vacuum pump. The extraction is repeated at least ten times till the extract is insensitive to SbCl_3/CHCl_3. The concentrated extract is allowed to separate over alumina column using 1% ethyl acetate in hexane and the fraction with \( \lambda_{\text{max}} \) at 350, 288, 275 nm is collected. This column purified fraction containing didehydroretinyl ester is vacuum evaporated and stored under inert conditions.
Column chromatography is performed over neutral alumina. Reactions are monitored by thin-layer chromatography. TLC plates of silica gel G are visualized either with UV lamp or in an iodine chamber. Reverse phase HPLC of the compounds are performed on a Supelcosil-LC8 (25 cm × 4.6 mm, 5 µm) column with a guard column of C18 materials using single channel at 350 nm. Retention time is reported in minutes and is compared with the standard sample. A gradient solvent system is used for this analysis. For pump A MeOH:H₂O (85:15) and for pump B MeOH: DCM (80:20) containing 10 mmol ammonium acetate and 0.01% acetic acid were used. The programme sets at 40 min. ¹H NMR chemical shifts (δ) are given with TMS (0 ppm) as internal standard.

DCM is distilled over CaCl₂ under an inert atmosphere. Hexane is freshly distilled. Unless stated otherwise, all reagents are purchased from commercial sources and used without additional purification. Diethyl ether is made peroxide free by distillation of the commercial sample kept in dark after addition of some iron powder in it. All the experiments from extraction to purification of final product are carried out in yellow light and under inert atmosphere.

3,4-Didehydroretinol. The concentrated purified didehydroretinyl ester extract is dissolved in 5 mL MeOH, followed by addition of 25 mL methanolic KOH (40%) and 50 µL of 0.02% BHT solution. The mixture is allowed to reflux for 30 min at 40-50°C. After cooling, it is diluted with H₂O and extracted with diethyl ether (peroxide free). The diethyl ether is concentrated and the resultant residue is purified over neutral alumina column. The saponified dehydroretinol is eluted with 5% diethyl ether in hexane. These procedures are repeated for 10 numbers of livers and pooled didehydroretinol (DROL) sample is used in the reaction. The purified and concentrated didehydroretinol extract is stored under inert conditions. Data for DROL: λₘₚₜ=341 nm; RP-HPLC τₘₚₜ=31.37 min; ¹H NMR (CDCl₃, 300 MHz): δ 7.37 (s, 1H), 6.67-5.69 (m, 12H), 4.30 (s, 2H), 2.35 (s, 3H), 2.32-0.80 (m, 27H); MS (+ES): Found: m/z 579 (M+12H)⁺, C₄₀H₆₆O₂ has a mass of 566. This may be due to some impurities or hydrogenation of the product during analysis and the sample was a column purified one.

3,4-Didehydroretinyl retinoate. In a dried two necked round bottomed flask equipped with magnetic flee RA (6 mg, 0.02 mmol), CDI (3.24 mg, 0.02 mmol), DROL (5.5 mg, 0.02 mmol) and catalytic amount of DMAP are placed. One neck is locked with one stopper attached with nitrogen balloon and the other neck is locked by another stopper. 20 mL freshly dried DCM is injected through the stopper to the reaction mixture. Then the temperature of the system is set to 0°C and the mixture is allowed to stir for 12 h. The solvent of the reaction mixture is then concentrated at a vacuum pump and the resultant residue is purified through neutral alumina column. The product didehydroretinyl retinoate (DRR) is eluted in 2-5% ethyl acetate in hexane (60.05 mg, 55%). Data for DRR: λₘₚₜ=353 nm; RP-HPLC τₘₚₜ=31.37 min; ¹H NMR (CDCl₃, 300 MHz): δ 7.37 (s, 1H), 6.67-5.69 (m, 12H), 4.30 (s, 2H), 2.35 (s, 3H), 2.32-0.80 (m, 27H); MS (+ES): Found: m/z 579 (M+12H)⁺, C₄₀H₆₆O₂ has a mass of 566. This may be due to some impurities or hydrogenation of the product during analysis and the sample was a column purified one.

**Results and Discussion**

The present work was initiated with the extraction of didehydroretinol ester from fish liver. The ester form is saponified with methanolic KOH solution and purified by preparative column chromatography. The purified didehydroretinol (DROL) is confirmed by HPLC. It gives a peak at retention time 15.71 min (Figure 1).

It is further supported by NMR data. The signal at δ 7.27 is due to extra conjugation in cyclohexene ring; δ 6.53-6.59 is due to conjugation system, δ 0.92-2.85 is due to cyclohexene ring. The concentration of purified DROL is calculated from UV-spectra using equation (1)

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\text{Concentration in g} = \frac{\text{OD} \times \text{TV}}{E_{1\%1cm} \times 100} \quad \text{(1)}
\]

where \( E_{1\%1cm} \) for DROL = 1445
After achieving purified DROL, retinoic acid (RA) is allowed to react with N,N-carbonyl diimidazole (CDI) in equimolar amount. The reaction mixture becomes reddish as time progresses and $\lambda_{\text{max}}$ of the reaction mixture shifts to 390 nm. At this moment DROL is added to the above reaction mixture in equimolar amount to that of RA along with catalytic amount of dimethyl amino pyridine (DMAP). The solvent DCM used in this reaction is dried over CaCl$_2$. Each and every apparatus used in this reaction is also air dried before use. The reaction is started at 0°C and is allowed to stir to RT (Scheme I). The formation of the product is checked by small TLC plates. After developing, the TLC plates are examined under UV lamp. The product is then purified by neutral alumina column. The purified product is analysed by reverse phase HPLC, NMR spectra and LCMS.

The product is reddish yellow in colour. It shows $\lambda_{\text{max}}$ absorption at 358 nm in UV spectrum. From this spectrum the yield of the product has been calculated by using equation 1. In RPHPLC chromatogram it shows a peak at retention time 31.37 min whereas RA gives a peak at 5.66 min and DROL at 15.71 min (Figure 1) with same condition of RPHPLC system. The peak corresponding to the product is a mix of two peaks which are inseparable which and may be due to formation of isomer of the product. So from RPHLC it has been confirmed that a new product is formed which has a different retention time than those of DROL and RA. A similar observation has also been noticed in developed TLC plates when co-chromatographed with standard DROL and RA. From the mass showed in LCMS (+ESI) it is further confirmed that a new derivative of RA is formed (Figure 2). The M+ peak is seen at $m/z$ 579. This may
be due to hydrogenation of some C=C double bond during different procedures.

**Conclusion**

In conclusion, the conversion of RA to a substituted RA product has been demonstrated with DROL. The process appears to be adaptable for synthesis of vitamin A derivatives by a simple procedure as it is very difficult to handle the retinoid compounds without being degraded. The reaction scheme has followed only one step and is a one pot procedure with lower risk of exposure to light and air. Use of easily available starting material, simple reaction conditions and good overall yield are some of the highlights of the procedure. The most important point to be noted is that the newly synthesized derivative DRR contains vitamin A acid and vitamin A\(_2\), both of which possess high pharmaceutical value. Further studies on the biochemical fate of DRR in animal models are necessary before employing this hybrid compound for human skin treatments.

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**References**