RIBOFLAVIN, A TESTOSTERONE 5α-REDUCTASE INHIBITOR

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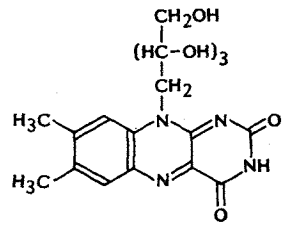
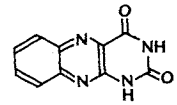
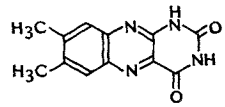
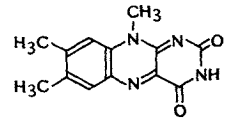
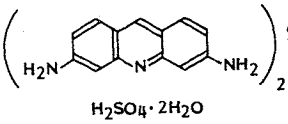
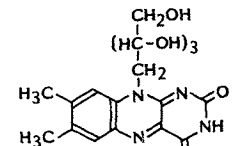
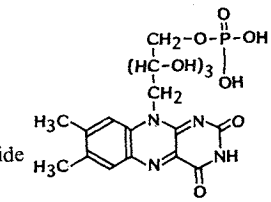
In the course of our search for inhibitors of testosterone 5α-reductase, we have previously reported about WS-9659 A and B, phenazine compounds[1]. We now wish to describe the effect of riboflavin from yeast on rat prostate 5α-reductase. A strain was cultivated in a 30-liter jar fermenter containing 20 liters of a medium consisting of glycerol 3%, soybean meal 1% corn steep liquor 0.5%, dried yeast extract 0.5%, $CaCO_3$ 0.2% (pH 6.5) for 3 days at 30°C under aeration of 20 liters per minute and agitation of 250 rpm. Testosterone 5α-reductase inhibition activity was assayed by the method we reported previously[1]. The whole broth (20 liters) was extracted with an equal volume of acetone at neutral pH, and filtered with diatomaceous aid (1 kg). After the filtrate was concentrated *in vacuo* to remove acetone, the active compound was adsorbed on a column of Dowex 1-X2 (OH^-, 1.5 liters, Dow Chemicals) at pH 7.0 and eluted with 2% CH_3COOH. The eluate was concentrated *in vacuo* and freeze-dried to give a crude powder. The powder was dissolved in water and passed through a column of Dowex 50W-X2 (H^+, 1.0 liter, Dow Chemicals) and developed with water. A biologically active component was further purified by preparative TLC (Kieselgel 60 F_{254}, Merck), and eluted with BuOH-CH_3COOH-H_2O (4:1:1). The Rf value of the active component was *ca.* 0.3 in this solvent system. The eluate was concentrated *in vacuo* and the resulting powder adsorbed on a column of Dowex 1-X2 ($HCOO^-$, 100 ml).

The column was washed with water and the active compound eluted with 2% HCOOH. The eluate was concentrated *in vacuo* and freeze-dried to give an orange powder (50 mg). This powder is soluble in alkaline solution, slightly soluble in lower alcohols and insoluble in ethyl acetate, ether, chlorinated and saturated hydrocarbons. The UV absorption spectrum of this compound, in alkaline solution, showed λ_{max} at 230, 270, 350 and 444 nm. The absorption maxima shifted to 222, 264 and 390 nm in acidic solution, suggesting the existence of a phenolic or enolic functional group. The active substance was identified as riboflavin (I) by IR spectrum, ^1H and



Riboflavin (I)

Table 1. Rat prostate testosterone 5α-reductase inhibition (T5α-RI) activities of selected compounds.

Drug	Structure	T5α-RI IC_{50} (M)
Alloxazine		9.4×10^{-6}
Lumichrome		9.7×10^{-6}
Lumiflavine		7×10^{-7}
Proflavine hemisulfate		9.3×10^{-7}
Riboflavin		5×10^{-6}
Flavin mononucleotide		5×10^{-6}

Alloxazine, lumichrome and lumiflavine were obtained from Sigma Chemicals. Proflavine hemisulfate and flavin mononucleotide were purchased from Nacarai Tesque.

^{13}C NMR spectra (data not shown). The concentration of riboflavin required to give IC_{50} was estimated from the titration curve to be 5×10^{-6} M. We also assayed other similar compounds such as lumichrome and lumiflavine. The results are shown in Table 1.

Riboflavin is a common product of several fungi and bacteria grown proper conditions[2,3].

It is used against riboflavin deficiency and hyperlipoproteinemia. It is reported here, for the first time, as a testosterone 5α-reductase inhibitor.

Also, riboflavin is a co-factor of many oxidoreductases, while testosterone 5α-reductase utilizes the pyridine nucleotide, NADPH, to reduce testosterone. It is interesting that the co-factor for flavin enzymes, riboflavin, inhibits pyridine linked enzyme reaction.

Among the riboflavin analogs tested, lumiflavine and proflavin hemisulfate were more active than riboflavin itself. Therefore, it may be worthwhile investigating the activity of other riboflavin analogs for increased testosterone 5α-reductase inhibition activity.

References

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3) ORTS, A.; A. RAMOS-CORMENZANA, F. RUIZ-BERRAQUERO & A. BAYA: Study of the synthesis and accumulation of vitamins in *Bacillus* species. ARS Pharm. 17: 285~294, 1976