A New Synthesis of Biopterin and L-Neopterin

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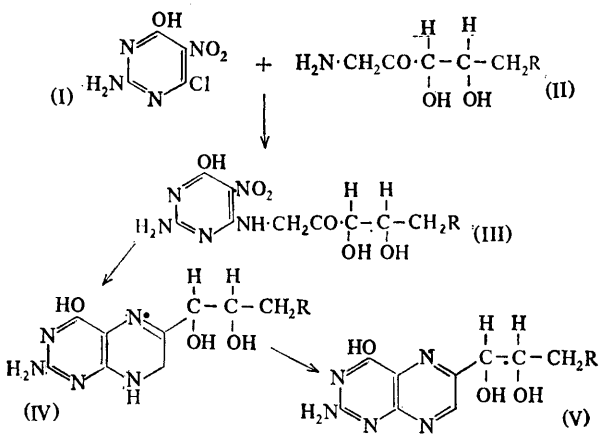
BIOPTERIN [2-amino-4-hydroxy-6-(L-*erythro*-1,2-dihydroxypropyl)pteridine] (V; R=H) and L-neopterin [2-amino-4-hydroxy-6-(L-*erythro*-1,2,3-trihydroxypropyl)pteridine] (V; R=OH) are growth factors for *Crithidia fasciculata*.[1,2] Several 5,6,7,8-tetrahydropteridine derivatives are co-factors for phenylalanine hydroxylase *in vitro*,[3] and a dihydrobiopterin is the naturally occurring co-factor for this enzyme in rat liver.[4] Biopterin has been isolated from human urine.[1]

The literature syntheses of biopterin[5,6] and L-neopterin[6,7] are based on the condensation of 2,4,5-triamino-6-hydroxypyrimidine with 5-deoxy-L-arabinose and L-arabinose, respectively.

This type of synthesis gives the compound with the side-chain in the 7-position as the main product, and a tedious chromatographic separation is required in order to obtain a small quantity of the 6-isomer. With the object of preparing enough of these pteridines for biological evaluation, we sought an unambiguous synthesis. In the course of work leading to the synthesis of xanthopterin, Stuart and Wood[8] treated 2-amino-4-chloro-6-hydroxy-5-nitropyrimidine with 1-amino-1-deoxy-D-*erythro*pentulose and reduced the resulting pyrimidinylaminoketose with hydrogen in the presence of Raney nickel. The reaction mixture at this stage had a



u.v. spectrum corresponding to a 6-alkyl-2-amino-7,8-dihydro-4-hydroxypteridine, and Stuart and Wood postulated the presence of the 6-(D-*erythro*-1,2,3-trihydroxypropyl) compound. In addition, Maclean *et al.*,[9] have noted that 5,6,7,8-tetrahydrobiopterin glucoside is converted into biopterin glucoside by manganese dioxide at pH 3.

We have prepared 1-amino-1-deoxy-L-*erythro*-pentulose (II; R=OH) from L-arabinose by Stuart and Wood's procedure for the corresponding D-compound. Treatment of this aminoketose with the chloropyrimidine (I) in aqueous ethanol, using sodium hydrogen carbonate as the proton acceptor, gave the nitropyrimidinyl-aminoketose (III; R=OH) in 34% yield. This nitro-compound was then hydrogenated in water, in the presence of Raney nickel, and the resulting solution showed peaks in the u.v. at 258 and 360 mμ, consistent with the dihydropteridine (IV; R=OH).[8] Manganese dioxide oxidation of this solution, adjusted to pH 3, at room temperature overnight, afforded an impure product, probably containing the 6-carboxylic acid. However, a similar oxidation at pH 5 and 0° overnight gave a product of high purity. In these experiments, the solids from the oxidation were extracted with dilute aqueous ammonia, the extract was evaporated and the residue crystallized from 20% aqueous acetic acid to give L-neopterin in 40% yield as pale yellow micro-rosettes, λ_{max} (0·1N-NaOH) 255 (24,000), 365·5 mμ (ε 7600); λ_{max} (0·1N-HCl) 248 (11,600), 321·5 mμ (ε 8020); λ_{max} (water) 275·5 (14,600), 348·5 mμ (ε 6180). Chromatography on phosphorylated cellulose paper, using 1/1 n-propanol–water as developing solvent, showed a single fluorescent spot (R_F 0·4).

In the synthesis of biopterin, it was first necessary to synthesize the unknown 1-amino-1,5-dideoxy-L-*erythro*-pentulose (II; R=H). 5-Deoxy-L-arabinose was prepared from L-rhamnose by a modification of the method described by Patterson *et al.*,[5] with an overall yield of 45%. In order to synthesize the required aminoketose from 5-deoxy-L-arabinose it was necessary to condense this with dibenzylamine and effect the Amadori rearrangement using diethyl malonate.[10] Hydrogenolysis of the dibenzylamino-sugar in the presence of palladium charcoal then gave the amino-ketose (II; R=H). The synthesis then followed the path outlined for L-neopterin, with similar results, and pure crystalline biopterin (V; R=H) was obtained without any chromatographic separation, λ_{max} (0·1N-NaOH) 254 (23,500), 365 mμ (ε 7300); λ_{max} (0·1N-HCl) 248 (11,450), 322 mμ (ε 8000). Chromatography on phosphorylated cellulose paper, using 1/1 n-propanol–water as developing solvent, showed a single fluorescent spot (R_F 0·6).

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