SYNTHESIS OF trans-5-(p-HYDROXYPHENYL)-4-AMINOISOXAZOLID-3-ONE — AN INHIBITOR OF THE ENZYMIC TRANSFORMATION OF TYROSINE

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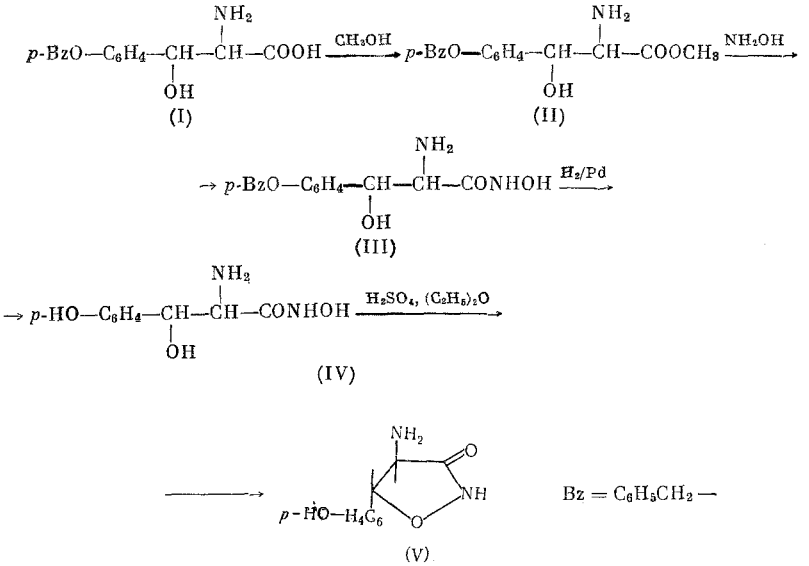
We have previously reported the synthesis of compounds of the substituted isoxazolid-3-one series, with the predicted properties of substrate-like inhibitors of pyridoxal enzymes [1-3]. A study of the mechanism of inhibition showed that these compounds, corresponding sufficiently close to the substrate portion of the active center of the enzyme, are capable of entering into the normal enzymic reaction, in one of the stages of which, opening of the isoxazolidone ring occurs and protein groups become blocked leading to inhibition of the enzyme [4-6].

During these studies it was of interest to obtain an inhibitor of the enzymic conversion of tyrosine, another important aminoacid. Since, in the case of phenylalanine, the trans form of 5-phenyl-4-aminoisoxazolid-3-one was shown to be the more active, it seemed expedient also to obtain specifically the trans isomer of 5-(p-hydroxyphenyl)-4-aminoisoxazolid-3-one, the corresponding inhibitor of tyrosine. None of the known methods of obtaining cyclic esters of hydroxamic acids were adaptable to the synthesis of this substance because of the instability of the hydroxyaryl serine being superimposed upon the lability of the isoxazolidone ring during the various transformations. The difficulties were successfully overcome by using the method suggested by us earlier for obtaining 5-phenyl-4-aminoisoxazolid-3-ones [3].



p-Hydroxyphenylserine esters have not been reported and all attempts to obtain them under the usual conditions for aminoacids led to decomposition of the starting material. Under the conditions found by us, we succeeded in obtaining (II) in ~50% yield. The hydroxamic acid (III) was obtained in the usual way and subsequent selective removal of the benzyl protecting group led to (IV). It was converted finally into (V) by gradual treatment with concentrated H_2SO_4 and ether (under conditions excluding sulfonation in the nucleus and the formation of an ether of the phenolic hydroxyl group).

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EXPERIMENTAL

Threo-β-(p-benzyloxy)phenylserine (I) was obtained; according to [7].

Threo-β-(p-benzyloxy)phenylserine Methyl Ester (Hydrochloride) (II). 2 g (I) was added gradually with stirring to 80 ml absolute methanol cooled to 0°, and containing an equivalent amount of dry hydrogen chloride. After complete solution of (I) and with stirring and cooling (temperature not above 0°), the clear solution was slowly saturated with dry hydrogen chloride until the appearance of a pale pink coloration. Afterwards the solution was set aside overnight at 5°, filtered and the solvent evaporated in vacuum at 10-15°. The mixture of crystals and oil remaining was treated again in the same way. After the repeat saturation of the methanol, the solution was set aside for a second day at 5°. After evaporation of the methanol, the residue was recrystallized from absolute ethanol. 1.4 g (58%) (II) was obtained with mp 165-166°. Found %: Cl 10.68. $C_{17}H_{20}O_4NCl$. Calculated %: Cl 10.51.

Hydroxamic Acid of Threo-β-(p-benzyloxy)phenylserine (III). To a solution of 1.7 g (II) hydrochloride in 15 ml absolute methanol sodium methoxide (0.140 g sodium in 2 ml absolute methanol) was added dropwise, the sodium chloride separated and hydroxylamine (0.480 g hydroxylamine hydrochloride, 0.180 g sodium, 2.6 ml methanol) and sodium methoxide (0.140 g sodium in 2 ml methanol) added, with stirring, to the filtrate. The solution was stirred 30 min at -5° to 0° and set aside overnight at room temperature. The solvent was evaporated in vacuum at 20°, the residue dissolved in 50 ml water and filtered. The filtrate was acidified with glacial acetic acid to pH 8 and set aside overnight at 0°. The precipitate of (III) which had separated was filtered off, washed with cold water then with a little ethanol and dried in vacuum; 1 g (66%) (III) was obtained with mp 150-151° (decomposition). Found %: N 4.45. $C_{16}H_{18}O_4N_2$. Calculated %: N 4.63.

Hydroxamic Acid of Threo-β-(p-hydroxy)phenylserine (IV). 0.5 g (III) was dissolved with slight warming in 75 ml absolute methanol, a solution of ammonia in methanol added to pH 9 and the mixture hydrogenated over 15 mg Pd until absorption of the theoretical amount of hydrogen. The catalyst was filtered off, and the clear filtrate concentrated to 15-20 ml-volume and diluted with 10 ml absolute ethanol. A day later the precipitate which separated was filtered off, washed with cold ethanol and dried in vacuum. 270 mg (80%) (IV) was obtained with mp 155-156° (decomposition). Found %: N 6.35. $C_9H_{12}O_4N_2$. Calculated %: 6.60.

trans-5-(p-Hydroxyphenyl)-4-aminoisoxazolid-3-one (V). To 0.212 g (IV), cooled to -10 to -15° and stirred continuously, was added dropwise 0.2 ml concentrated H_2SO_4 cooled to -15°. The mixture was stirred below 0° until solution, then added dropwise to 70 ml absolute ether cooled to -10 to -15°. The yellow oil which separated was washed several times with cold absolute ether and then treated with a solution of ammonia in alcohol (3 × 15 ml). The combined extract was concentrated at reduced temperature to 2-3 ml-volume (pH 6) and set aside overnight at 0°. The precipitate which had separated was filtered off, washed with small portions of cold absolute ethanol and absolute ether and dried in vacuum. 50 mg (26%) (V) was obtained with mp 135-140° (decomposition). R_f = 0.1 (n-butanol-water-acetone-concentrated ammonia, 8 : 6 : 1 : 1) revealed with 0.2% ninhydrin in acetone solution or 4% sodium nitroprusside solution. Found %: C 55.38; H 5.04. $C_9H_{10}O_3N_2$. Calculated %: C 55.66; H 5.19.

CONCLUSIONS

Trans-5-(p-hydroxyphenyl)-4-aminoisoxazolid-3-one, an inhibitor of the enzyme transforming tyrosine, has been synthesized.

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