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ENZYMATIC SYNTHESIS OF AZA-L-TRYPTOPHANS: The Preparation of 5- and 6-Aza-L-tryptophan[1]

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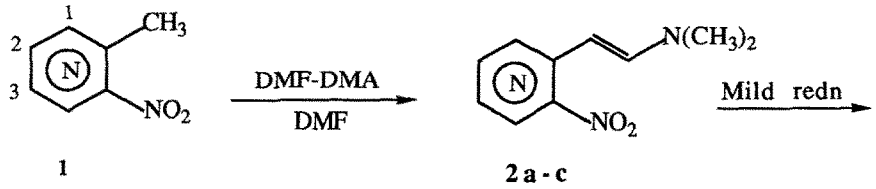
Abstract:
Enzymatic reaction of the azaindoles **3a-d** with tryptophan synthase [EC. 4.2.1.20] and L-serine results in synthesis of aza analogs of tryptophan **4a-d.** The synthesis of **4b** and **4c** has not been previously reported. The azaindoles **3a-c** were prepared from nitropicolines **1a-c.**

The essential amino acid tryptophan is vital to the normal function of all living organisms. It serves a central role in the biosynthesis and metabolism of proteins, peptides, enzymes, hormones, alkaloids, and other biologically important products. 7-Azatryptophan has shown a wide range of biological activities in a number of systems[2-6]. 7-Azatryptophan is a competitive antagonist of tryptophan metabolism, and has been used to study enzyme mechanisms[7]. It has been synthesized both chemically[8] and enzymatically[9,10] and is commercially available in racemic form. However, there have been few studies of other aza analogs of tryptophan[6,9,11-13]. Consequently, the potential of other aza analogs of tryptophan to possess significant biological activity is of considerable interest. We report here a convenient synthesis of these aza analogs, and describe the first reports of 5- and 6-aza-L-tryptophan. Our method also provides for the preparation of 4-aza-L-tryptophan and improved yields of 7-aza-L-tryptophan[9]. Thus, we have prepared 4-, 5-, 6-, and 7-aza-L-tryptrophan in one step from the corresponding azaindole with L-serine and recombinant tryptophan synthase[14]. The absence of C-2 and C-3 proton coupling in the NMR spectrum in each of the isomers prepared clearly indicates condensation on carbon at C-3, rather than on nitrogen as observed with indazole[13].

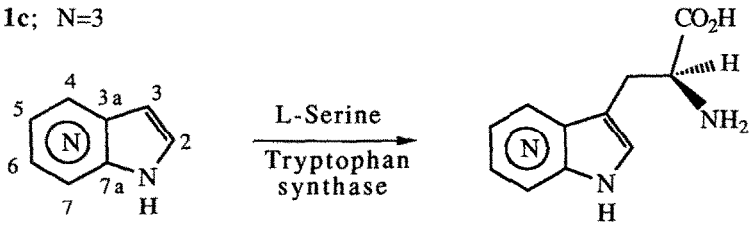
7-Azaindole (Aldrich) was obtained commercially, and 6, 5, and 4-azaindole were prepared by modifying the methods reported by Yakhontov et al. [15,16], and by Dormoy and Heymes[17]. Condensation of *ortho*-nitropicolines **1a-c** with N,N-dimethylformamide dimethylacetal (DMF-DMA) gave the enamines **2a-c** which upon reduction gave **3a-c.**

SCHEME 1



1 2a-c

1a; N=1
1b; N^{+}–O^{-}= 2
1c; N=3



3 4a-d

3a; N=4
3b; N=5
3c; N=6
3d; N=7

The reported catalytic hydrogenation and Raney nickel-hydrazine reduction of the enamines **2a-c** did not give satisfactory results in our hands. These methods afforded fair amounts of uncyclized byproducts in which the olefin bond was reduced prior to the nitro group. A milder and more selective method using catalytic hydrogen transfer was found to give much higher yields and cleaner products[18]. Five percent palladium on carbon in a formic acid-methanol mixture or aqueous ammonium formate was used to effect cyclization to the azaindoles **3a-c**. Aqueous ammonium formate was found to be more suitable than formic acid-methanol, since the workup was facilitated by sublimation of the ammonium carbonate byproduct during the course of the reaction. The azaindoles were then added to a reaction mixture of L-serine and tryptophan synthase[19]. Isolated yields of 61-88% were obtained after 2-11 days[20]. We are presently investigating the reactivity of each azaindole in the reaction catalysed by tryptophan synthase.

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19. A typical reaction mixture consisted of azaindole (16.9 mM), L-serine (22.8 mM), pyridoxal phosphate (53 mM), potassium phosphate buffer (100 mM, pH 8), NaCl (16.2 mM), tryptophan synthase (2000-4000 units), and NaN_3 (3 mM). The reaction mixture was incubated in the dark at 37°C. Progress was monitored by TLC, and the reaction was stopped by heating in a water bath at 80°C for approx. 30 min. to denature the protein. The precipitated protein was

removed by filtration or centrifugation, and the solution concentrated *in vacuo*. Isolation of **4a-c** was effected by applying the solution over reverse phase silica gel, eluting with 15% aqueous methanol (150 mL).

20. 4-Azatryptophan **4a** Isolated yield of 53 mg (61%) from 50 mg of 4-azaindole, after 2-3 days; $[\alpha]^{25}_D$ +10.7° (c 0.5, 1N HCl); IR(nujol) 3307cm^{-1}(br), 3137, 1684, 1622, 1590, 1558, 1506, 1404, 1333, 1306, 1234, 1158, 1133, 1114, 973, 889, 849, 761; ^{1}H NMR (D_2O/DCl, 300.13 MHz, acetone δ 2.06 ppm) 8.41 ppm(d, 1H, J=8.6Hz, 7-ArH), 8.35(d, 1H, J=5.9Hz, 5-ArH), 7.92(s, 1H, 2-ArH), 7.53(dd,1H, J=5.9Hz, J=8.4Hz, 6-ArH), 4.35(t, 1H, J=6.5Hz, αCH), 3.50(dd, 1H, J=15.9Hz, J=6.5Hz, βCH), 3.42(dd, 1H, J=15.9Hz, J=6.5Hz, βCH); ^{13}C NMR (D_2O/DCl, 75.46 MHz, acetone δ 30.6 ppm) 171.75 ppm(C=O) 136.36(5-C) 135.03(3a-C or 7a-C) 134.21(6-C) 133.18(7a-C or 3a-C) 129.19(7-C) 117.22(2-C) 103.06(3-C) 53.03(αCH) 24.43(βCH$_2$); Anal for $C_{10}H_{11}N_3O_2 \cdot H_2O$ Calcd %C 53.81, H 5.87, N 18.82; Found %C 54.26, H 5.51, N 18.74. 5-Azatryptophan **4b** Isolated yield of 202 mg (77.5%) from 150 mg of 5-azaindole after 10-11 days; $[\alpha]^{25}_D$ +21.6° (c 0.5, 1N HCl); IR (nujol) 3122cm^{-1}(br), 1609(s), 1576, 1541(s), 1540, 1400, 1337, 1318, 1236, 1207, 1166, 1091, 1028(s), 881, 822, 811, 656.3; ^{1}H NMR (D_2O/DCl, 250.13 MHz, D_2O 4.65 δ ppm) 8.86 ppm(s, 1H, 4-ArH), 8.10(d, 1H, J=6.7Hz, 6-ArH), 7.72(d, 1H, J=6.7Hz, 7-ArH), 7.56(s,1H, 2-ArH), 3.99(t, 1H, J=6.0Hz, αCH), 3.34(d, 2H, J=6.0Hz, βCH); ^{13}C NMR (D_2O/DCl, 62.89 MHz,) 173.86 ppm(C=O) 134.78(4-C) 131.97(6-C) 130.90(7-C) 130.15(7a-C or 3a-C) 124.21(3a-C or 7a-C) 111.61(3-C) 109.83(2-C) 54.88(αCH) 26.22(βCH$_2$); Anal for $C_{10}H_{11}N_3O_2 \cdot 2.5H_2O$ Calcd %C 47.99, H 6.59, N 16.79; Found %C 47.35, H 6.52, N 16.04. 6-Azatryptophan **4c** Isolated yield of 337 mg (88%) from 200 mg of 6-azaindole after 6-7 days; $[\alpha]^{25}_D$ +25.0° (c 0.615, 1N HCl); IR (nujol) 3385cm^{-1}(br), 3121, 1620, 1567, 1421, 1400, 1377(s), 1363, 1346, 1314, 1282, 1246, 1136, 1113, 1090, 1067, 1044(s), 966(s), 917(s), 899, 835, 769; ^{1}H NMR (D_2O/DCl, 300.13 MHz, acetone δ 2.06 ppm) 8.80 ppm(s, 1H, 7-ArH), 8.02 (d, 1H, J=6.6Hz, 5-ArH), 7.92(d, 1H, J=6.6Hz, 4-ArH), 7.93(d,1H, J=1.0Hz, 2ArH), 4.24 (t, 1H, J=6.1Hz, αCH), 3.41(d, 2H, J=6.1Hz, βCH); ^{13}C NMR (D_2O/DCl, 75.46 MHz) 172.81 ppm(C=O) 139.35(7-C) 137.73(5-C) 132.32(7a-C or 3a-C) 128.77(4-C) 128.02(3a-C or 7a-C) 116.40(2-C) 110.53(3-C) 54.47(αCH) 25.99(βCH$_2$); Anal for $C_{10}H_{11}N_3O_2 \cdot 2H_2O$ Calcd %C 49.78, H 6.22, N 17.42; Found %C 49.78, H 6.20, N 17.39. 7-Azatryptophan **4d** Isolated yield of 30.6 mg (88%) from 20 mg of 7-azaindole after 3-4 days; $[\alpha]^{25}_D$ +14.7° (c 0.275, 1N HCl); ^{1}H NMR (D_2O/DCl, 300.13 MHz, acetone δ 2.06 ppm) 8.43 ppm(dd,1H, J=8.0Hz, J=1.0Hz, 6-ArH), 8.13(dd, 1H, J=6.0Hz, J=1.0Hz, 4-ArH), 7.41(s, 1H, 2-ArH), 7.33(dd, 1H, J=8.0Hz, J=6.0Hz, 5-ArH), 3.95(t, 1H, J=6.20Hz, αCH), 3.27(d, 2H, J=6.20Hz, βCH$_2$); ^{13}C NMR (D_2O/DCl, 75.46 MHz, acetone δ 30.6 ppm) 173.87 ppm(C=0) 139.27(7a-C or 3a-C) 136.53(6-C) 133.76(5-C) 128.58(4-C) 125.47(3a-C or 7a-C) 115.77(2-C) 109.76(3-C) 54.74(α-CH) 26.03(β-CH$_2$).