THE SYNTHESIS OF THE COOKED-BEEF MUTAGEN 2-AMINO-1-METHYL-6-PHENYLIMIDAZO-[4,5-b]PYRIDINE AND ITS 3-METHYL ISOMER

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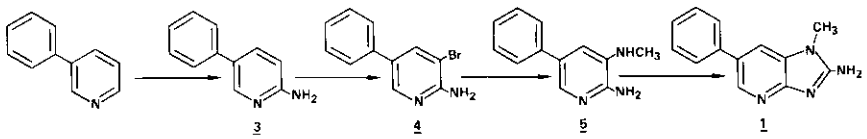
Abstract —— 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, a mutagenic compound newly isolated from cooked beef, and its 3-methyl isomer have been synthesized. The spectroscopic data and the mutagenicity of the isomers are compared.

The presence of mutagens in cooked proteinaceous foods has been reported by many investigators[1,2] and for the past several years the isolation and identification of the mutagenic compounds has been the focus in several laboratories (see Miller[3] for review). Often, a unique structural prediction from the spectroscopic data derived from the isolated mutagens is not possible, and proof of structure necessitates the unambiguous synthesis of isomers. Furthermore, the synthesis of larger amounts of identified potent mutagens is necessary for short-term toxicological and genetic assessment. With these tests the health risks associated with the consumption of mutagens in our diet can begin to be estimated. The newly identified mutagen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), is the most mass-abundant mutagen found thus far in fried beef[4], and therefore, is of prime importance for further toxicological studies.

These syntheses have been carried out to 1) confirm our proposed structure for the isolated mutagen[5], and 2) to make sufficient quantities for further investigation of its biological effects.

The synthesis of PhIP 1 began with the commercially available 3-phenylpyridine (25 g) which was aminated at the 6 position with sodium amide (12.6 g) in toluene by the Chichibabin reaction under conditions similar to those used by Tsuji et al[6]. The product was extracted with chloroform and crystallized from benzene to give 3 (16 g, 58%, mp 130°C (lit[6]. 131-132°C)).

SCHEME I



3 4 5 1

The aminoderivative 3 (4 g) was brominated in acetic acid for 1 h at ambient temperature and crystallized from ethanol to produce 2-amino-3-bromo-5-phenylpyridine 4 (3.1 g)[7]. The bromine of 4 (1.0 g) was then substituted with a methylamino group by heating a solution of $CuSO_4$ (10 mg) in 40% aqueous methylamine in a sand bath at 200°C in a PTFE-lined pressure bomb for 72 h. The product was extracted with chloroform to produce 2-amino-3-methylamino-5-phenylpyridine 5. The diamine 5 is air sensitive and was handled in a nitrogen atmosphere. For the cyclization of 5 to 1, the dried material was transferred with a small amount of ethanol to a pressure bomb containing cyanogen bromide. This was then sealed under a nitrogen atmosphere and heated 1 h at 175°C. After cooling, the solvent was evaporated, the residue dissolved in chloroform and extracted into aqueous HCl (pH = 2). The water was evaporated and the residue purified by flash chromatography[8] (chloroform-methanol 10:1 v/v) to yield 1 as a white residue (82 mg, 9% yield from 4). Other cyclization conditions were tried, such as those used by Grivas and Olsson[9], but no reaction occurred.

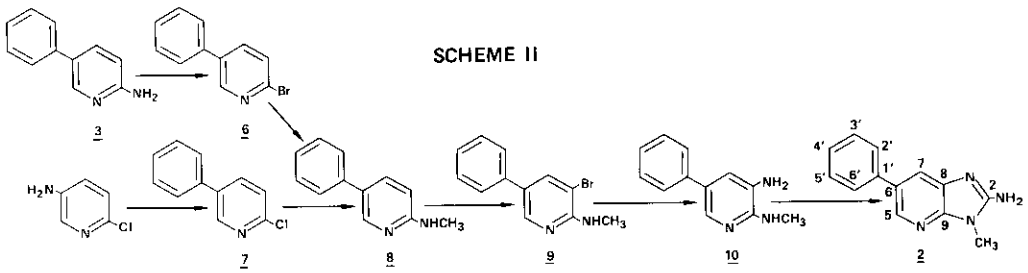
The synthesis of 2-amino-3-methyl-6-phenylimidazo[4,5-b]pyridine (2) was accomplished by either of two routes (scheme II). Using the method of Mikhaiov[10] for the substitution of 2-aminopyridine, 2-amino-5-phenylpyridine 3 (3.0 g) was dissolved in 48% HBr (10.75 ml) at -5°C, and bromine (3.3 g) was added followed by 12 M HCl (4.4 ml). Aqueous sodium nitrite (4.8 g) was added dropwise and stirred 1 h at -5°C, then 40% sodium hydroxide (10.7 ml) was added, the reaction mixture was allowed to warm to room temperature and was extracted with chloroform to yield 2-bromo-5-phenylpyridine 6[11] (1.4 g, 34%). Compound 6 (1.4 g) was then allowed to react with methylamine using the same procedure used for compound 4, with heating for 48 h to produce 2-methylamino-5-phenylpyridine 8[12] (0.98 g, 89%).

An alternative route to 8 utilizes the commercially available 3-amino-6-chloropyridine and a diazotization reaction to make 2-chloro-5-phenylpyridine. The Gomberg reaction of Adams et al.[13] gave only a 1% yield in our laboratory, however, a new method by Stavenuiter et al.[14] using pentyl nitrite to diazotize the amine gave compound 7[15] in 61% yield after silica column chromatography with chloroform. The reaction of 7 (1.0 g) with aqueous methylamine then displaced the chlorine to yield the 2-methylamino-5-phenylpyridine 8 (0.88 g, 90%).

Nitration of 8 with nitric acid in sulfuric acid gave the 4'-nitro instead of the desired 3-nitro derivative. However, bromination of 8 (0.88g) in acetic acid produced 2-methylamino-3-bromo-5-phenylpyridine (9) (1.1 g, 89%)[16]. The bromoderivative 9 (1.0 g)

was then allowed to react with 58% NH_4OH, under similar conditions used for the bromine substitution of 4, to produce 3-amino-2-methylamino-5-phenylpyridine (10). This was cyclized with cyanogen bromide, purified in the same manner as was done for 1 and crystalized from 2-propanol to yield compound 2 (54 mg, 6.3% yield from 9) as white needles.

SCHEME II



A comparison of the chemical, physical and biological properties of the two isomers is shown in Table I.

Table I

Comparison of the Properties of PhIP and its 3-Methyl Isomer

	PhIP	3-Methyl Isomer of PhIP
UV (CH_3OH): λ_{max}(log ε)	225(4.46), 273(4.00), 316(4.46)	240(4.31), 312(4.14)
IR (KBr): cm^{-1}	3091, 1666, 1595, 1551, 1472 1439, 1423, 1408, 1269, 760	3327, 3306, 3063, 1676, 1562, 1493, 1414, 1358, 762, 698
^1H NMR (200.07 MHz δ DMSO-d_6)	8.31 (5-H,d,J=2.1 Hz) 7.71 (7-H,d,J=2.1 Hz) 7.72 (2',6'-H,d,J=7.3 Hz) 7.48 (3',5'-H,t,J=7.3 Hz) 7.35 (4'-H,t,J=7.3 Hz) 7.0 (NH_2,broad s) 3.60 (1-CH_3,s)	8.15(5-H,d,J=2.1 Hz) 7.64(7-H,d,J=2.1 Hz) 7.66(2',6'-H,d,J=7.3 Hz) 7.45(3',5'-H,t,J=7.3 Hz) 7.33(4'-H,t,J=7.3 Hz) 6.9 (NH_2, broad s) 3.55(3-CH_3,s)
^{13}C NMR δ (50.31 MHz, DMSO-d_6) (decoupled)	28.43(me-c), 111.84(7-c), 126.32(2',6'-c), 126.49(4'-c) 127.77(6-c), 128.74(3',5'-c) 138.79(1',8-c), 139.02(5-c) 155.84(2-c), 157.81(9-c)	27.01(me-c), 118.29(7-c) 126.68(2',4',6'-c), 128.79(3',5'-c) 129.69(6-c), 135.92(8-c) 136.01(5-c), 139.01(1'-c) 147.72(9-c), 156.63(2-c)
MS: m/z (calculated for $C_{13}H_{12}N_4$, 224.1062)	224.1068	224.1073
Melting point	327-328°C	217°C
HPLC separation: K' (Spherisorb NH_2 column hexane/n-propanol/acetic acid, 60:40:0.1 v/v)	5.21	1.0
Salmonella mutagenicity (strain 1538)	431 revertants/nmole	5.0 revertants/nmole

The UV absorbance spectra are distinctive for each isomer. The 3-Methyl isomer has its major absorbance maximum shifted to a shorter wavelength and is lacking the absorbance peak at 273 nm. The IR spectra are similar in general but differences in band shifts and intensities are observed. A comparison of the ^{1}H NMR spectra show the largest difference in the peak shift of H-5 on the pyridine ring at 8.31 for the PhIP and at 8.15 for the 3-methyl isomer but with differences in the other protons as well. The ^{13}C NMR spectra show differences in chemical shifts between the two isomers the largest being the expected shift of carbon 9. Tentative assignments for all carbon atoms were based on substituent shifts and intensities in corresponding standard compounds. The carbon numbering system is shown in Scheme II for compound 2. The mass spectra are not distinguishable, showing similar fragments and intensities with ions at m/z 224(rel. int. 100, M), 223(77), 225(31), 140(31), 127(26), 196(21), 154(19), 170(18), 115(15), 77(12). It is interesting to note that the melting points of the two compounds differ by 110°C. The two phenylimidazopyridines can be easily separated using normal-phase HPLC. The biological activity of the two isomers differs also greatly with the PhIP being 86 times more mutagenic in the Ames/Salmonella test with activation by Aroclor induced rat liver S-9.[17,18]

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11. MS, m/z (rel. int.):154(100), 233(85), 235(72), 127(68), 128(28), 126(18), 153(18), 155(14), 234(11), 236(9).

12. MS, m/z (rel. int.):184(100), 156(85), 155(70), 154(57), 183(58), 127(38), 128(26), 115(26), 141(23), 77(18). ^{1}H NMR (DMSO-d6):δ8.33 (6-H,d,J=2.3 Hz), 7.70 (4-H,dd,J=8.7, Hz, J=2.3 Hz), 7.56 (2',6'-H,d,J=7.6 Hz), 7.39 (3',5'-H,dd,J=7.6 Hz, J=7.3 Hz), 7.25 (4'-H,t,J=7.3 Hz), 6.59 (NH, broad s) 6.54 (3-H,d,J=8.7 Hz), 2.81 (N-CH_3,d,J=4.8 Hz).

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15. MS, m/z (rel. int.):189(100), 191(44), 127(41), 154(40), 190(22), 128(18), 126(17), 153(13), 188(11), 152(8); ^{1}H NMR(DMSO-d6):δ8.73 (6-H,d,J=2.4 Hz), 8.15 (4-H,d,J=8.5 Hz), 7.73 (2',6'-H,d,J=8.1 Hz), 7.59 (3-H,d,J=8.5 Hz), 7.51(4'-H,t,J=7.3 Hz), 7.47 (3',5'-H,dd,J=7.6 Hz,J=7.3 Hz).

16. MS, m/z (rel. int.): 264.0097(100), calculated for $C_{12}H_{11}N_2Br$, 264.0085, 262(99), 234(86), 236(66), 235(40), 154(38), 263(37), 233(33), 127(26), 140(20); ^{1}H NMR(DMSO-d_6):δ8.43 (6-H,d,J=2.1 Hz), 8.29 (4-H,d,J=2.1 Hz), 7.68 (2',6'-H,d,J=6.7 Hz), 7.46 (3',5'-H,dd,J=7.0 Hz,J=6.7 Hz), 7.36 (4'-H,t,J=7.0 Hz), 5.93 (N-H, broad s), 2.99 (N-CH_3,d,J=4.8 Hz); mp 232-239°C.

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