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MANNICH REACTION OF 5-HYDROXY-β-CARBOLINE. APPLICATION TO THE SYNTHESIS OF NOVEL OXAZINOPYRIMIDOCARBOLINE DERIVATIVES

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Abstract – 5-Hydroxy-β-carboline **4** was synthesized and submitted to the Mannich reaction towards formaldehyde and some primary amines. It undergoes an original reaction of *bis*-aminomethylation-cyclisation giving the oxazinopyrimidocarboline derivatives **5a-d**. The structure of these compounds was confirmed by their high-resolution mass, IR, ^{1}H NMR and ^{13}C NMR spectral data.

INTRODUCTION

Recently, carbolines have received much interest due to their biological and pharmaceutical importance. For example, in the case of β-carbolines, many investigations have been reported on their antitumor activity[1-5] and their variety of actions on the central nervous system.[6-8] Moreover, many natural products contain this heterocyclic system as harmine,[9] a β-carboline alkaloid isolated from *Peganum harmala L.* which has shown a strong cytotoxicity to tumor cell lines *in vitro*, 1-methoxycanthin-6-one[10] isolated from the medicinal plant *Ailanthus altissima swingle* described for its proapoptotic activity and its synergism with agents that induce tumor cell death (Figure 1). On the other hand, N-substituted dihydro-1,3-oxazines condensed with aromatic rings have various pharmacological properties. For example, a number of oxazinoindoles **1** have been reported to possess diuretic, antihypertensive, anti-inflammatory, antiulcerous, sedative and analgesic activities in rats and mice.[11] Also; the oxazinocarbazoledione derivative **2** has been reported to possess a strong cytotoxic activity against colon cells and pulmonary carcinoma cells.[12]

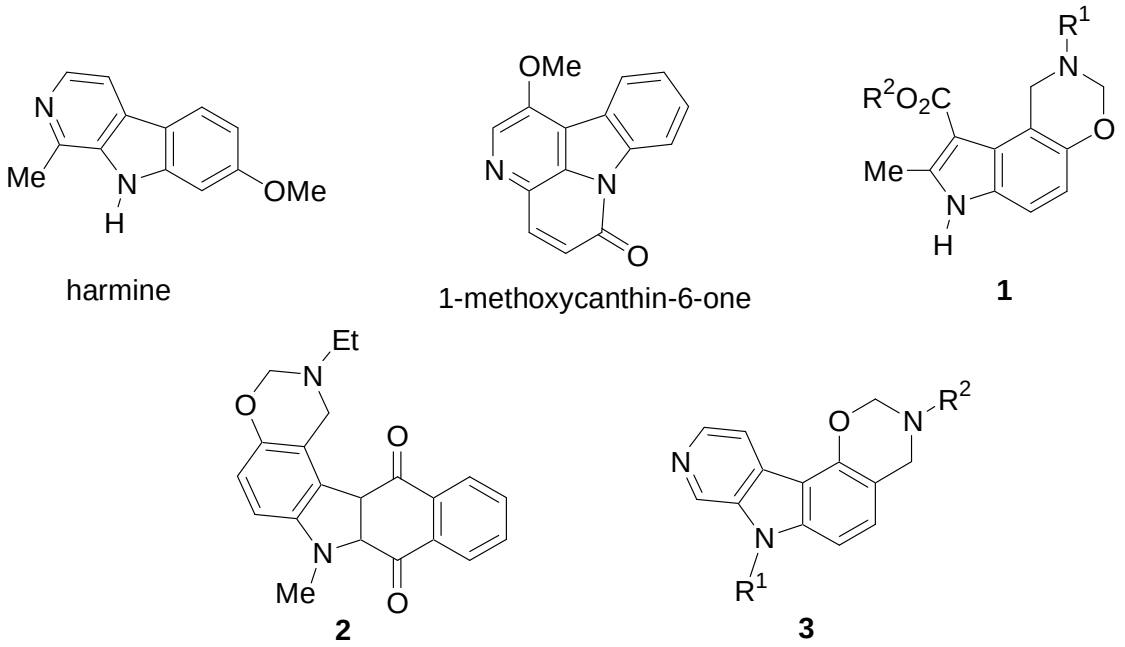
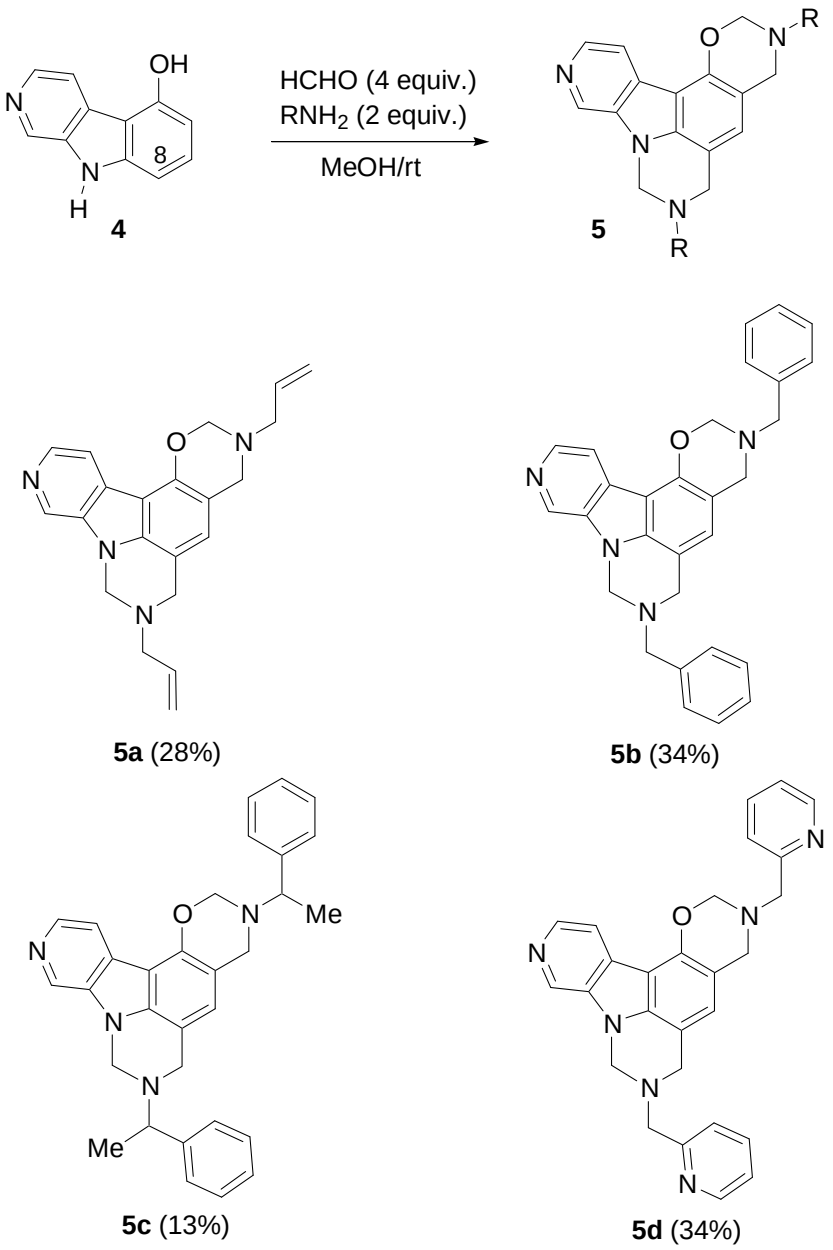


Figure 1

RESULTS AND DISCUSSION

As part of our investigation[13] on the biological properties of fused carbazole and carboline derivatives, we planned to synthesize a series of oxazinocarbolines **3** through a normal Mannich reaction employing three components: 5-hydroxy-β-carboline **4**, primary amine and formaldehyde. According to literature, aminomethylation of phenols always occurs at the position *ortho* to the hydroxyl group, even if the *para* position is unoccupied.[14] On the other hand, primary amines can react to give various products, like secondary amines, dihydrooxazines or *bis*-hydroxyaryl-amines, depending upon the reaction conditions. Additionally, it is known that polyfunctional substrates may produce a variety of cyclic compounds. In this paper, we report the results of our preliminary work starting from hydroxycarboline **4**. This latter was prepared starting from *meta*-anisidine as previously reported by Quéguiner *et al*.[15]

The reaction of **4** with formaldehyde (2 equiv.) and primary amine (1 equiv.) in methanol at rt yielded a small amount of the oxazinopyrimidocarboline **5** via a Mannich-type *bis*-aminomethylation followed by intramolecular cyclization by formaldehyde. The expected oxazinocarbolines **3** were not observed. Optimization of reaction conditions was made by using 4 equivalents of formaldehyde and 2 equivalents of primary amine. However, the yields of compounds **5** remain moderate but a substantial amount of the starting compound **4** was recovered (Scheme 1).



Scheme 1

Concerning the regiochemistry, *bis*-aminomethylation may occur on the positions *ortho* and *para* to the hydroxyl group or on the *ortho* position to the hydroxyl group and at the heterocyclic NH group. In either case, the *bis*-aminomethylated compound reacts with two equivalents of formaldehyde. The resulting intermediate may react intramolecularly with the phenolic oxygen to form the oxazine ring and with the indolic N atom or the C-8 atom to form the tetrahydropyrimidine ring.

This unexpected transformation constitutes a novel example of Mannich reaction of phenolic substrate, affording at the same time the oxazine and the diazine rings. Consequently it constitutes a convenient and simple strategy to synthesize in one-pot, these new pentacyclic carboline derivatives. Similar pentacyclic ring systems reported in the literature have shown potent biological activity. Among them,

pyrimidocarbazole ER-37328[16] exhibits a strong tumoricidal activity both *in vitro* and *in vivo* against solid tumor cells, and substituted pentacyclic carbazolones **6**[17] have been described as allosteric modulators at muscarinic acetylcholine receptors (Figure 2).

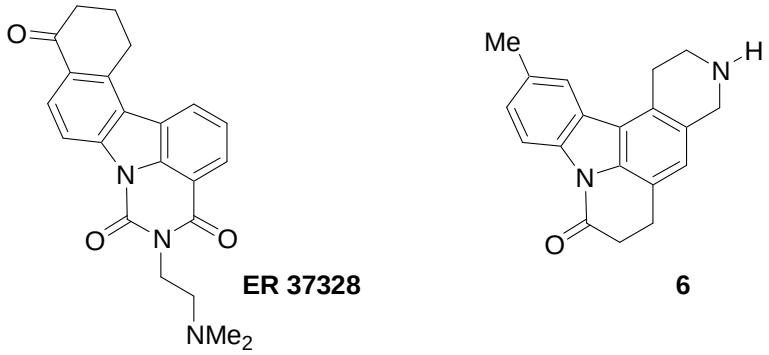


Figure 2

In summary, some novel oxazinopyrimidocarboline derivatives have been synthesized by a one-pot Mannich reaction of 5-hydroxy-β-carboline with primary amines and formaldehyde. Further studies to extend this transformation to other primary amines and other heterocyclic phenols as 5-hydroxy-α-carboline and 5-hydroxycarbazole, are in progress.

EXPERIMENTAL

1. General: Melting points were measured with a Büchi apparatus (capillary tube). The IR spectra were recorded with a Perkin-Elmer 1310 spectrophotometer. The NMR spectra were recorded with a Bruker AM 300 spectrometer (^{1}H-NMR: 300 MHz, ^{13}C-NMR: 75 MHz). Chemical shifts (δ) are reported in ppm using tetramethylsilane (TMS) as an internal reference. Coupling constant (*J*) values are given in Hz. High-resolution mass spectra (HRMS) were recorded on a ThermoFinnigan MAT 95 XL spectrometer.

2. General procedure for the Mannich reaction: A 37% aqueous solution of formaldehyde (560 μL, 6.52 mmol) in MeOH (10 mL) was cooled in an ice bath. The corresponding primary amine (3.26 mmol) was added and the resulting mixture was stirred at 0 °C for 30 min. Compound **4** (300 mg, 1.63 mmol) in MeOH (10 mL) was then slowly added. After complete addition, stirring was continued at rt for a variable amount of time, the evolution of the reaction being followed by TLC. After evaporation of the solvent, the residue was purified by column chromatography on silica gel.

3,7-Diallyl-3,4,7,8-tetrahydro-2*H*,6*H*-1,3-oxazino[6,5-*g*]pyrimido[5,6,1-*jk*]-β-carboline (5a): Purification by column chromatography on silica gel with CH_2Cl_2/MeOH (95:5) yielded **5a** in 28% yield.

mp 116 °C. IR (KBr): 2923, 1626, 1499 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ ppm 8.74 (d, 1H, *J* = 0.9 Hz, H-10), 8.42 (d, 1H, *J* = 5.3 Hz, H-13), 8.07 (dd, 1H, *J* = 0.9 and 5.3 Hz, H-12), 8.07 (s, 1H, H-5), 5.90 (m, 2H, $CH_2\underline{CH}=CH_2$), 5.28-5.00 (m, 4H, $CH_2CH=\underline{CH_2}$), 5.19 (s, 2H, H-2), 5.10 (s, 2H, H-8), 4.15 (s, 2H, H-6), 4.11 (s, 2H, H-4), 3.48 (d, 2H, *J* = 6.4 Hz, $\underline{CH_2}CH=CH_2$), 3.12 (d, 2H, *J* = 6.4 Hz, $\underline{CH_2}CH=CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 49.7, 50.4, 54.7, 55.9, 62.3, 82.8, 118.3, 119.0, 117.4, 124.1, 131.1, 134.9, 135.3, 139.1, 108.0, 109.7, 110.9, 127.3, 137.4, 135.5, 149.7. HRMS (CI): calcd for $C_{21}H_{23}N_4O$ ($M+H^+$) 347.1872, found 347.1879.

3,7-Dibenzyl-3,4,7,8-tetrahydro-2*H*,6*H*-1,3-oxazino[6,5-*g*]pyrimido[5,6,1-*jk*]-β-carboline (5b): Purification by column chromatography on silica gel with AcOEt/MeOH (90:10) yielded **5b** in 34% yield. mp 190 °C. IR (KBr): 2924, 1633, 1591 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ ppm 8.69 (s, 1H, H-10), 8.46 (d, 1H, *J* = 5.3 Hz, H-13), 8.15 (d, 1H, *J* = 5.3 Hz, H-12), 7.41-7.23 (m, 10H, H aromat.), 6.81 (s, 1H, H-5), 5.17 (s, 2H, H-2), 5.15 (s, 2H, H-8), 4.19 (s, 2H, H-6), 4.12 (s, 2H, H-4), 4.05 (s, 2H, $\underline{CH_2}$Ph), 3.65 (s, 2H, $\underline{CH_2}$Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 49.3, 50.4, 55.7, 56.9, 62.2, 83.0, 117.4, 124.3, 127.7, 127.5, 128.5 (2C), 128.6 (2C), 129.0 (2C), 129.3 (2C), 131.1, 139.1, 108.0, 109.7, 111.0, 127.3, 135.1, 137.4, 137.8, 138.3, 149.7. HRMS (CI): calcd for $C_{29}H_{27}N_4O$ ($M+H^+$) 447.2185, found 447.2186.

3,7-Bis(*alpha*-Methylbenzyl)-3,4,7,8-tetrahydro-2*H*,6*H*-1,3-oxazino[6,5-*g*]pyrimido[5,6,1-*jk*]-β-carboline (5c): Purification by column chromatography on silica gel with AcOEt/MeOH (90:10) yielded **5c** in 13% yield. mp 125 °C. IR (KBr): 2972, 1631, 1531 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.58 (s, 1H, H-10), 8.42 (d, 1H, *J* = 5.3 Hz, H-13), 8.09 (d, 1H, *J* = 5.3 Hz, H-12), 7.36-7.14 (m, 10H, H aromat.), 5.68 (s, 1H, H-5), 5.22 (d, 1H, *J* = 12.2 Hz, H-2), 5.02 (d, 1H, *J* = 12.2 Hz, H-2), 4.20 (s, 2H, H-8), 4.02 (s, 2H, H-6), 3.98 (s, 2H, H-4), 3.39 (s, 2H, $\underline{CH}$(Me)Ph), 1.3 (s, 3H, *J* = 6.52 Hz, CH($\underline{Me}$)Ph), 1.5 (s, 3H, *J* = 6.52 Hz, CH($\underline{Me}$)Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 21.86, 22.07, 48.7, 49.3, 61.4, 81.1, 58.1, 58.5, 117.8, 124.5, 127.7 (2C), 127.8 (2C), 128.0, 128.9 (2C), 129.0 (2C), 129.5, 131.1, 139.1, 108.2, 110.5, 111.3, 112.6, 128.9, 129.9, 138.5, 140.8, 144.4. HRMS (CI): calcd for $C_{31}H_{31}N_4O$ ($M+H^+$) 475.2498, found 475.2500.

3,7-Bis(pyridin-2-ylmethyl)-3,4,7,8-tetrahydro-2*H*,6*H*-1,3-oxazino[6,5-*g*]pyrimido[5,6,1-*jk*]-β-carboline (5d): Purification by column chromatography on silica gel with CH_2Cl_2/MeOH (90:10) yielded **5d** in 34% yield. mp 122 °C. IR (KBr): 2923, 1633, 1592 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ ppm 8.70 (s, 1H, H-10), 8.61 (dd, 2H, *J* = 4.3 Hz, H-6'), 8.42 (d, 1H, *J* = 5.3 Hz, H-12), 8.11 (d, 1H, *J* = 5.3 Hz, H-13), 7.69-7.18 (m, 6H, H aromat.), 6.80 (s, 1H, H-5), 5.23 (s, 2H, H-2), 5.17 (s, 2H, H-4), 4.22 (s, 4H, H-6 and H-8), 4.14 (s, 2H, $\underline{CH_2}$Py), 4.02 (s, 2H, $\underline{CH_2}$Py). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 50.4, 51.1,

57.9, 59.1, 63.5, 83.8, 117.8, 123.1, 123.3, 123.5, 123.7, 124.8, 131.4, 137.0, 137.1, 139.4, 150.0, 150.2, 108.5, 110.1, 111.3, 127.7, 135.1, 137.8, 158.2, 158.3, 158.8. HRMS (CI): calcd for $C_{27}H_{25}N_6O$ ($M+H^+$) 449.2090, found 449.2094.

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