SYNTHESIS OF AN ANTIBACTERIAL AND ANTIFUNGAL CINNOLINE DERIVATIVE BY REARRANGEMENT OF A β-CARBOLINE DERIVATIVE

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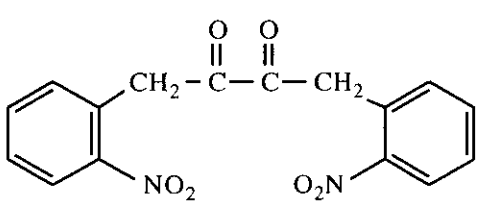
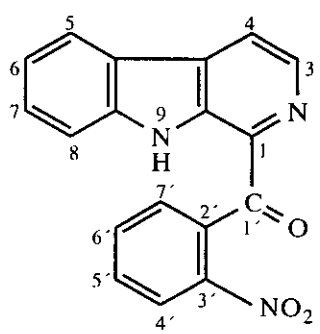
Abstract- A heterocyclic system (**6**) containing a β-carboline moiety is described. *o*-Nitrophenylpyruvic acid and tryptamine hydrochloride were condensed to give 1-(2′-nitrobenzyl)-1,2,3,4-tetrahydro-β-carboline (**3**). **3**, on dehydrogenation, gave 1-(2′-nitrobenzyl)-β-carboline (**4**) which was treated with methanolic KOH to furnish 1-(3′,2′,1′-benzisoxazole)-β-carboline (**5**) which on thermolysis or photolysis afforded the indolocinnoline derivative (**6**).

In our preceding paper we have reported that anthranilopapavarine, when subjected to either pyrolysis or photolysis, rearranges to a isoquino [1,2-*b*]quinazoline derivative.[1] In continuation of our efforts to develop generalized and efficient methodologies for the synthesis of target heterocycles, it was thought worthwhile to use the above finding to synthesize indolic heterocycles. In this paper we describe the synthesis of the new cinnoline derivative (**6**). Cinnoline derivatives have been reported to be associated with antimicrobial activities[2] and compound (**6**) also showed interesting antimicrobial properties.

The synthesis of **6** was achieved in a four-step sequence (Scheme 1). Thus condensation of tryptamine hydrochloride with *o*-nitrophenylpyruvic acid by using the modified method of Harley-Mason[3] afforded 1-(2′-nitrobenzyl)-1,2,3,4-tetrahydro-β-carboline (**3**). Another compound of formula $C_{16}H_{12}N_2O_6$ (HRms observed 164.0352, calcd for 164.03764) was isolated during the purification of compound (**3**). It showed uv absorption (λ_{max} 209, 260 nm), the appearance of a carbonyl band at 1735 cm^{-1}, aromatic absorptions (1618 and 1580 cm^{-1}) and the absence of OH and NH bands indicated it to be derived from *o*-nitrophenylpyruvic acid. The ^{1}H-nmr spectrum showed resonances for two methylene protons as a singlet at δ 4.28. Two triplets resonated at δ 7.59 and δ 7.44 for the two aromatic protons, while the remaining aromatic protons resonated as two doublets at δ 7.37 and δ 8.10. On the basis of spectroscopic evidences structure **1** was assigned to the compound $C_{16}H_{12}N_2O_6$.

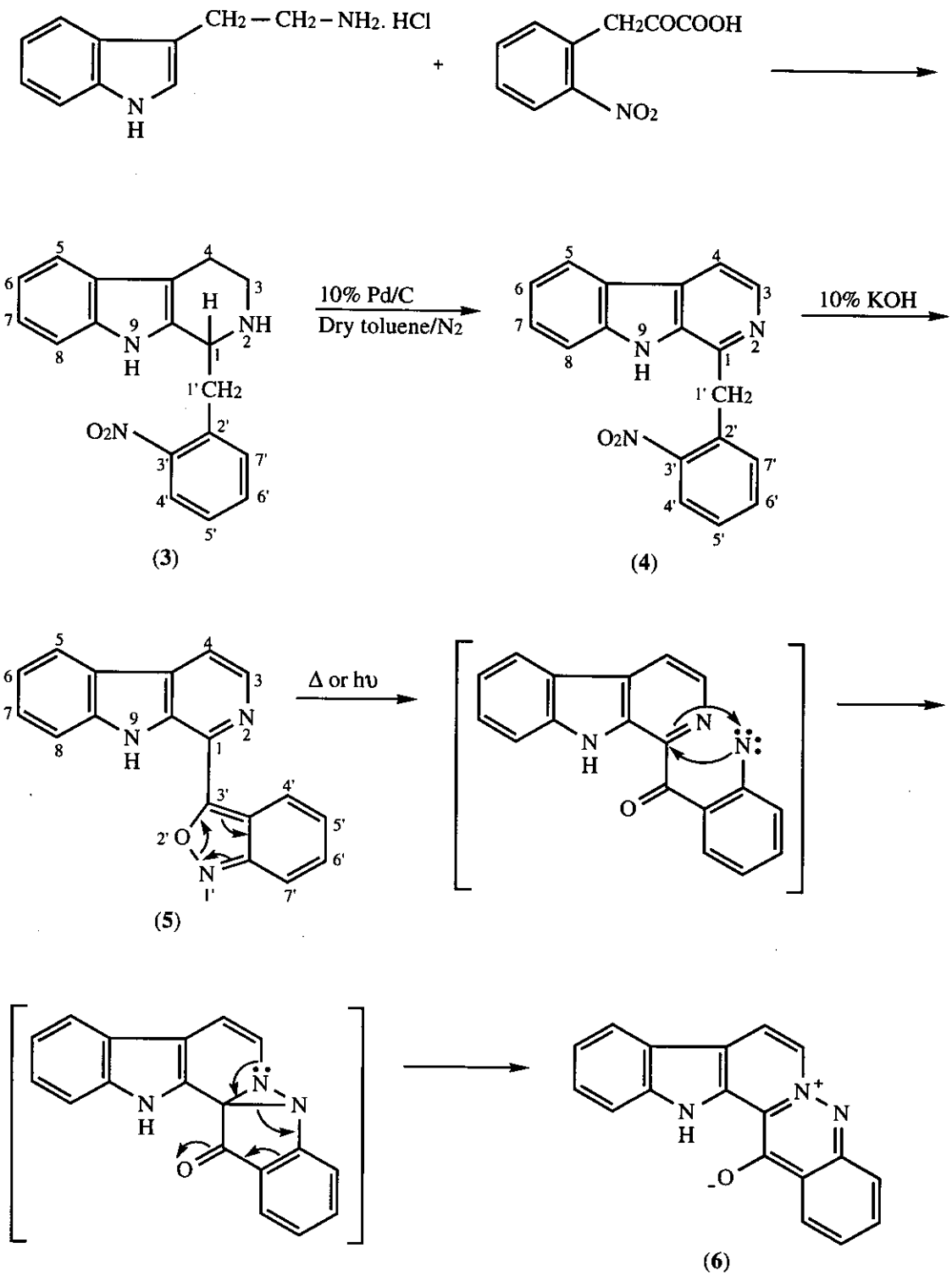


(**1**)



(**2**)

An alternative method involving the condensation of 2-nitrophenylacetyl chloride with tryptamine and attempted cyclization of the resulting amide with $POCl_3$ did not furnish the desired (**3**) in our case, also.[4]


CH2 — CH2 – NH2. HCl
CH2COCOOH
NO2
10% Pd/C
Dry toluene/N2
10% KOH
Δ or hυ
(3)
(4)
(5)
(6)

Scheme 1

The structure of **3** was supported by its uv spectrum (λ_{max} 200, 224, 273 nm), the appearance of two NH band in the ir spectrum (3350, 2900 cm^{-1}) and HRms (observed 307.1295 calcd for 307.1320). The ^{1}H-nmr spectrum showed resonances for 3 methylene protons between δ 2.75 - δ 3.60. Other assignments are shown in Table **1**. Compound (**3**) when heated under nitrogen in refluxing dry toluene with 10% Pd/C gave 1-(2′-nitrobenzyl)-β-carboline (**4**). Even under inert reaction conditions one of the major by-products was **2** formed during the reaction presumably by autooxidation which takes place during work-up. Compound (**2**) showed a distinct carbonyl absorption at 1635 cm^{-1} and the absence of a methylene group in the ^{1}H-nmr spectrum. Its HRms corresponded to the molecular formula $C_{18}H_{11}N_3O_3$. The compound was therefore identified as 1-(2′-nitrobenzoyl)-β-carboline (**2**). Other methods of dehydrogenation with DDQ, MnO_2 or sulphur did not furnish the desired **4**.

The structure of **4** was established by the following spectroscopic data. The ir spectrum showed only one NH absorption (3280 cm^{-1}) while the uv spectrum (λ_{max} 338, 289, 235, 212 nm) indicated a more conjugated system than that of **3**. The ^{1}H-nmr spectrum showed only one methylene group as a singlet at δ 5.33. Other assignments are indicated in Table **1**. By heating under reflux in 10% methanolic KOH, **4** gave the benzisoxazole (**5**) in 35% yield. The uv spectrum of compound (**5**) indicated a highly conjugated system (λ_{max} 260, 269, 287, 307, 322, 334, 411, nm), and its ir spectrum showed a peak for NH (3282 cm^{-1}). The mass spectrum showed the M^+ at m/z 285.0909 corresponding to the molecular formula $C_{18}H_{11}N_3O$. Its ^{1}H-Nmr spectrum did not show any methylene protons. The NH appeared as a broad singlet at δ 9.80.

Compound (**5**) when subjected to thermolysis gave the cinnoline derivative (**6**) in 60% yield as a bright yellow crystalline compound, having the formula $C_{18}H_{11}N_3O$ established by HRms. No dehydrorutaecarpine was formed. It appears that when methoxy groups are present on the benzene ring, it favours the acridone type rearrangement[5] while in the absence of the methoxy groups, the mesoionic inner salt formation is favoured.[6] Structure (**6**) was established from the following spectroscopic data. The ir spectrum showed an absorption peak at 3300 cm^{-1} due to the NH group. The absence of any carbonyl absorption ruled out the rutaecarpine-type structure. The uv spectrum showed bands at λ_{max} 218, 293, 357, 404, 461 indicating a highly conjugated system. Its mass spectrum afforded the molecular ion as the base peak at m/z 285.0892 in agreement with the formula $C_{18}H_{11}N_3O$ which was confirmed by peak matching. Other peaks appeared at m/z 257, 229, 167, 142 and 140. The ^{1}H-nmr assignments and COSY interactions are shown in Figure **1**.

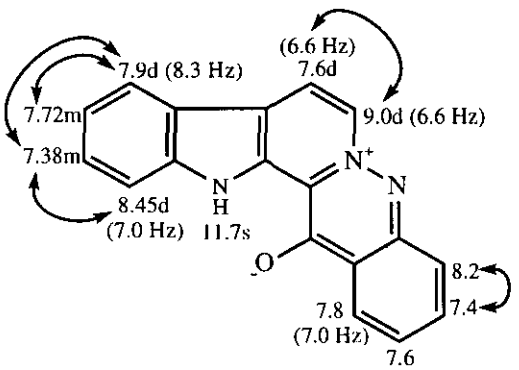


Figure 1: COSY 45° interactions in **6**.

BIOLOGICAL ACTIVITIES

Compound (**6**) was tested for antifungal activity in 200 μg/ml concentration against *Plevrotus ostreatus* (a), *Nigrospora oryzae* (b), *Microsporum canis* (c), *Epidermophyton floccosum* (d), *Drechslera rostrata* (e) and *Aspergillus niger* (f). It showed good activity against the organisms (a), (b) and (c), moderate activity against (d) and low activity against (e) and (f). It was found to be non-cytotoxic when the brine shrimp

assay[7] was performed in 1 x 10^{-3} µg/ml concentration. It did not show antibacterial activity against several pathogenic bacteria.

EXPERIMENTAL

Melting points were determined on a Buchi 510 melting point apparatus and are uncorrected. The yields were not maximized. Uv spectra were recorded in methanol solution on Shimadzu 240 spectrophotometer. The ir spectra were measured as potassium bromide pellets with JASCO IRA-1 spectrophotometer. Mass spectra were taken on Finnigan MAT 112 and Finnigan MAT 312 double focussing mass spectrometers connected to DEC PDP 11/34 computer systems. High resolution mass measurements were carried out by peak matching, using PFK as internal standard. ^{1}H-Nmr spectra were recorded in $CDCl_3$ on Bruker AM 300 and Bruker AM 400 FT-NMR spectrometers at 300 and 400 MHz and ^{13}C-nmr at 75 and 100 MHz respectively. Alumina E. Merck Art 1097 was used for column chromatography. E. Merk Kieselgel Si F254 was used for analytical and preparative tlc. Irradiation was carried out in a photochemical reaction unit RH 400-low for 1hr. at room temp. in the presence of Pyrex filter.

1,4-(2′-Nitrophenyl)-2-3-butanedione (1)

Purification of compound (**3**) by column chromatography using benzene as eluent afforded **1** (110 mg, 9%) mp 142°C. Uv λ_{max} (log ε) = 209 (4.42), 260 (4.05). Ir (KBr) cm^{-1} 1735, 1618, 1580, 1520,1350, 1072, 738, 715. ^{1}H-Nmr ($CDCl_3$) (δ ppm) δ 4.28 (s, 4H, 2 x CH_2), δ 7.37 (d, J = 7.6 Hz, 2H, H-6′), δ 7.44 (t, J = 8.1, 1.45 Hz, 2H, H-4′), δ 7.59 (t, J = 7.5, 1.3 Hz, 2H, H-5′) δ 8.10 (d J = 8.1 Hz, 2H, H-3′); HRms: Found 164.0352 (M^{2+}), calcd for $C_{16}H_{12}N_2O_6$, 164.0376; ms: m/z (rel.int.): 164 (M^{2+}, 90), 136 (80), ; 120 (34), 92 (40), 78 (100), 65 (21). *Anal.* Calcd for $C_{16}H_{12}N_2O_6$: C, 58.51; H, 3.68; N, 8.50. Found: C, 58.74; H, 3.56; N, 8.34.

1-(2′-Nitrobenzoyl)-β-carboline (2)

Compound (**2**) was also isolated in the dehydrogenation reaction as a by-product with a greater Rf value than compound (**4**). Compound (**2**) (130 mg, 32%) mp 136°C. Uv λ_{max} (log ε) = 197 (4.29), 219 (4.44), 28.7 (4.037), 289 (3.62). Ir (KBr) cm^{-1}. 3400 (NH), 1635 (CO), 1610, 1560 (aromatic), 1350, 1240, 1200, 960, 750. ^{1}H-Nmr (Table **1**); HRms: Found 317.0792 calcd for $C_{18}H_{11}N_3O_3$, 317.08003; FDms (100%); ms: m/z (rel. int.): 317 (15), 217 (24.5), 149 (8), 81 (42), 55 (100). *Anal.* Calcd for $C_{18}H_{11}N_3O_3$: C, 68.11; H, 3.46; N, 13.25. Found: C, 67.83; H, 3.36; N, 12.92.

1-(2′-Nitrobenzyl)-1,2,3,4-tetrahydro-β-carboline (3)

Tryptamine hydrochloride (1.2 g, 6.12 mmol.) was added to a solution of *o*-nitrophenylpyruvic acid (1.2 g, 5.74 mmol.) in 20% aqueous ethanol (25 ml). The reaction mixture was heated on a steam bath for 6 h, cooled, basified with conc. NH_4OH and extracted with CH_2Cl_2 (3 x 25 ml) the extract was washed with water, dried over Na_2SO_4, filtered and the solvent removed in vacuo to dryness (0.75 g). Purification by column chromatography with benzene as an eluent ultimately furnished three major products (**1-3**) together with unreacted tryptamine. Compound (**3**) (300 mg, 17%) mp 158°C (benzene). Uv λ_{max} (log ε) = 200 (4.52), 224 (4.63), 273 (4.07). Ir (KBr) cm^{-1} 3350, 2900 (NH), 1600, 1570 (aromatic), 1500, 1435, 1350, 1300, 1110, 960, 850, 775, 570. ^{1}H-Nmr (Table **1**); HRms: Found 307. 1295 (M^+), calcd for $C_{18}H_{17}N_3O_2$, 307.1320; FDms 307 (100%); ms: m/z (rel. int.) 307 (M^+, 8.4), 279 (5.3), 206 (16.6), 171 (100), 150 (10), 149 (87.7), 91 (53.1), 85 (18.5), 83.1 (15.8), 81 (16.9), 77 (13.9), 71 (34), 70 (19.4), 69 (26.7), 67 (15.6), 65 (21.6), 57 (51.92), 56 (17.4), 55 (44). *Anal.* Calcd for $C_{18}H_{17}N_3O_2$: C, 70.35; H, 5.53; N, 13.71. Found: C, 70.02; H, 5.41; N, 13.38.

1-(2′-Nitrobenzyl)-β-carboline (4)

To a solution of **3** (410 mg, 1.33 mmol.) in 50 ml of toluene (dry) was added Pd/C (0.5 g, 10%) and the mixture refluxed under nitrogen for 6 h. The solution was filtered and the solvent removed in vacuo. It

was separated on preparative tlc plates using chloroform as solvent to give compound (**4**) (100 mg, 24.7%); Uv λ_{max} (log ε) = 235.6 (2.5), 289 (4.1), 338.4 (4.5). Ir (KBr) cm^{-1} 3280 (NH), 1616, 1560 (aromatic) 1480, (rel. int.) 1420, 1380, 1300, 1220, 1140, 1070, 820, 740. ^{1}H-Nmr (Table **1**); HRms: Found 303.1008 (M^+), calcd for $C_{18}H_{13}N_3O_2$ 303.10077; FDms (100); ms: m/z, 303 (100), 287 (21), 286 (94), 271 (9), 257 (87), 256 (60), 243 (13), 228 (12), 197 (21), 167 (26), 149 (17), 140 (23), 128 (83), 114 (43), 60 (46), 55 (44).

1-(2′,1′-Benzisoxazol-3′-yl)-β-carboline (5)

To a solution of KOH (0.5 g, 1.75 mmol.) in methanol (5 ml) was added compound (**4**) (100 mg, 0.33 mmol) and the solution refluxed on a water bath for 2.5 h, cooled and diluted with dist. water (20 ml). After extraction of the diluted solution with $CHCl_3$ (3 x 20 ml) the total extract was washed twice with water and dried over Na_2SO_4, filtered and the solvent removed in vacuo. The resulting residue was separated by column chromatography with benzene as an eluent. Compound (**5**) was obtained as pure crystalline compound (35 mg, 35%); mp 260°C; uv λ_{max} (log ε): 198 (3.98), 260 (4.08), 269 (4.15), 387 (3.8), 307 (3.8), 322 (3.8), 334 (3.8), 411.6 (4.09). Ir (KBr) cm^{-1}: 3280 (NH) 1616, 1560 (aromatic) 1480, 1420, 1420, 1380, 1300, 1220, 1140, 1070, 820, 740. ^{1}H-Nmr (Table **1**), HRms: Found 285.0909 (M^+) calcd for $C_{18}H_{11}N_3O$ 285.0902; FDms: (100%); ms: m/z (rel. int.): 285 (100), 257 (29), 256 (29) 229 (15), 213 (7), 167 (18), 142 (21), 140 (25), 122 (16), 115 (17), 105 (44), 57 (50), 55 (45).

Thermolysis product (6)

The benzisoxazole (**5**) (35 mg, 0.12 mmol) was heated under N_2 at 260-270°C in a metal bath for 5 min. The resulting residue was dissolved in CH_2Cl_2 and separated on tlc using benzene and chloroform (1:1) as eluent. The rearranged compound (**6**) was obtained (21 mg, 60%). Uv (λ_{max} log ε): 204 (3.89), 220 (4.129), 405 (2.137). Ir (KBr) cm^{-1} 3300, 1610, 1584, 1570, 1540, 1460, 1420, 1380, 1290, 1260, 1235, 117, 1150, 760. ^{1}H-Nmr (Figure **1**); HRms: found 285.089 (M^+) calcd for $C_{18}H_{11}N_3O$, 285.0902, FDms: (100%); ms: m/z (% rel.int) 285 (100) M^+, 256 (10.6), 167 (4.6), 142 (6), 140 (6). *Anal.* Calcd for $C_{18}H_{11}N_3O$: C, 75.81; H, 3.87; N, 14.74. Found: C, 75.52; H, 3.81; N, 14.46.

Table 1: ^{1}H-NMR Data for Compounds (**2-5**).

	2	3	4	5
H	δ Mult (J Hz)	δ Mult (J Hz)	δ Mult (J Hz)	δ Mult (J Hz)
1	-	4.30 m	-	-
2	-	1.64 s	-	-
3	8.39 d (4.9)	2.75 m, 3.25 t (5.2)	8.38 d (6.4)	8.67 d (4.8)
4	8.10 d (4.9)	2.75 m, 3.10 t (5.2)	7.90 d (7.1)	8.05 d (4.8)
5	7.65 m	7.48 m	8.18 d (8.0)	8.17 d (7.9)
6	7.65 m	7.58 t (7.5)	7.49 t (7.6)	7.41 t (7.2)
7	7.65 m	7.43 m	7.30 t (7.8)	7.64 m
8	7.65 m	7.48 m	7.30 t (7.8)	7.64 m
9	10.35 s	8.07 br s	7.90 d (7.1)	7.64 m
1′	-	3.10 m (a), 3.6 (b)	5.33 s	-
2′	-	-	-	-
4′	8.16 t (7.8)	8.05 d (8.0)	7.68 m	7.40 m
5′	7.36 m	7.17 t (7.1)	7.68 m	7.17 t (6.6)
6′	7.81 t (7.4)	7.12 t (7.0)	7.68 m	7.30 t (6.6)
7′	8.22 d (7.4)	7.34 d (8.0)	7.68 m	8.68 m

Photolysis of 5

The benzisoxazole (**5**) (20 mg, 0.07 mmol) was subjected to uv irradiation in methanol with a photochemical reactor unit fitted with a low power uv lamp for 1 h at room temperature in the presence of a Pyrex filter. Evaporation of the solvent and separation of the resulting residue gave **6** (5 mg, 20%), as the only isolable product.

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