7-Azacinnolin-4(1*H*)-one Preparation and NMR Studies of Tautomery

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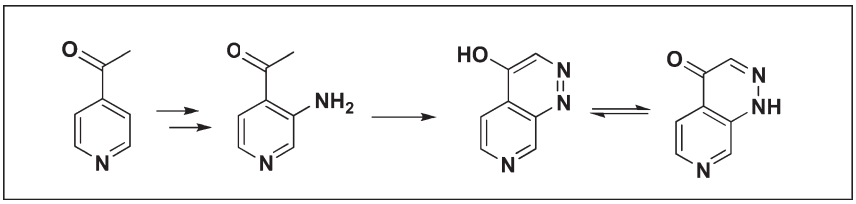

As part of our current investigations of nitropyridines, we hereby report the preparation of a new annulated heterocycle by C-azo coupling. Thus, the azacinnoline, pyrido[3,4-c]pyridazin-4(1*H*)-one (38%), was prepared from 4-acetyl-3-aminopyridine via diazotization. ^{1}H, ^{13}C, and ^{15}N NMR spectroscopic investigations revealed that the azacinnoline exclusively exists in the NH-keto tautomeric form in DMSO-d_6, CD_3OD, and D_2O.

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INTRODUCTION

Investigations on the chemistry of nitropyridines are currently in progress in our laboratories, based on the fact that a number of substituted 3-nitropyridines have become readily available through an improved nitration method [1,2]. We have previously reported the preparation of the new pyrido[3,4-c]thieno[3,2-e]pyridazine (**I**, X = S, Scheme 1) by diazo-coupling [3]. The furan and pyrrole products (**I**, X = O, NH) were also prepared in a similar manner [4]. The results demonstrated that the diazonium intermediate pathway readily allows the synthesis of pyridine-fused azo-coupling compounds from nitropyridines.

Pyridopyridazines (**II**) have been studied for preventing and treating atherosclerosis [5] and such heterocycles are also used as substrates for the preparation of antiviral agents [6]. Furthermore, other pyridazine compounds are biologically active, and the pyridazine moiety is incorporated in a series of pharmaceuticals. Cinnolines (**III**, Scheme 1) [7] are important intermediates in the preparation of the antidepressant binodaline [8] and the antibiotic cinoxacin [9]. A series of substituted benzo[c]cinnolines (**IIIa**) show herbicidal activity [10], whereas others are mutagenic substances [11], being identified as organic aza-heterocyclic pollutants [12]. The corresponding *N*-analogous pyrido[3,4-*c*]cinnoline (**IIIb**) ring structure has also been reported [13]. Cinnolin-4-ol (**IVa**) is used as a drug intermediate [14] and is a precursor for the preparation of potential antimalarial drugs and herbicides [15]. Recently, a detailed NMR study on the tautomerism of the benzo-fused heterocycle **IVa** has been reported [16]. It was concluded that this compound exists as the NH-keto isomer **IVb** in DMSO-d_6.

In contrast to the well-studied cyclization reaction to afford the cinnoline **IVb** (Scheme 1) by diazotization and C-azo-coupling, the corresponding preparation of the novel pyridine-analogue **2** (Scheme 2) has received hardly any attention. An early investigation reported an unsuccessful ring-closure of 4-acetyl-3-aminopyridine (**5**) after diazotization [17]. Applying either acidic or basic conditions, no products, arising from cyclization, could be observed or isolated.

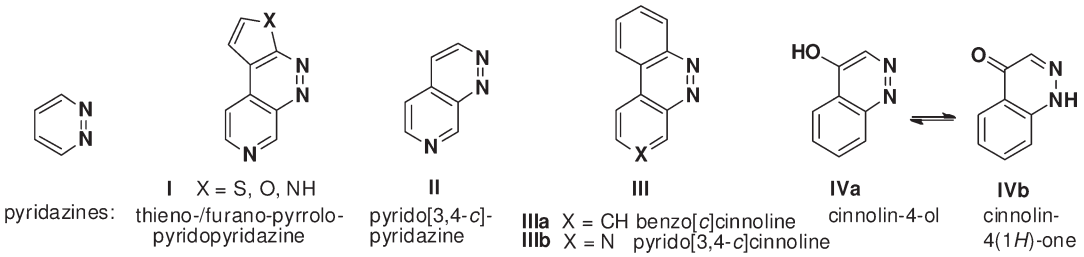
Because of the potential biological activity, the therapeutic use and the generally interesting properties of cinnolines, we wanted to prepare the new and previously unknown 7-azacinnolin-4-(1*H*)-one (**2**) (Scheme 2), being a pyridine-analogue of the benzo-fused cinnoline **IVb** (Scheme 1). The 4-substituted pyridyl diazonium salt **1** is readily prepared from the appropriate nitropyridine **4** via pyridylamine **5**. The following cyclization of diazonium intermediate **1** by intramolecular C-azo-coupling has been investigated, and the successful preparation of aza-cinnoline derivative **2** is hereby reported. The tautomerism of azacinnoline (**2**/**2′**) has been studied by ^{1}H, ^{13}C, and ^{15}N NMR spectroscopy.

RESULTS AND DISCUSSION

The three-step synthesis for the preparation of azacinnoline **2** is shown in Scheme 2.

The nitropyridine derivative **4** is reported to be available in 75% yield by nitration of 4-acetylpyridine (**3**),

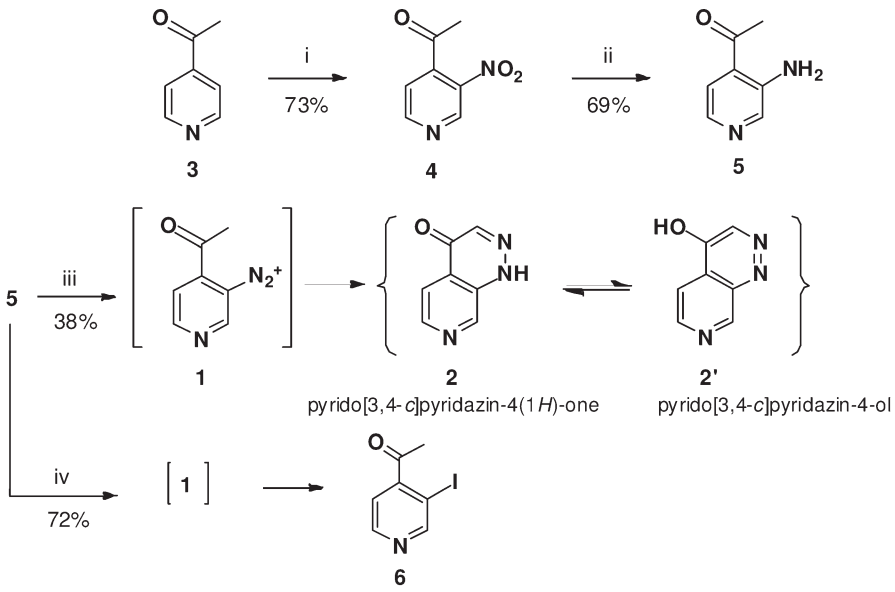
Scheme 1



using dinitrogen pentoxide (N_2O_5, DNP) as the nitrating agent in liquid SO_2. The reaction mixture is poured into water before work-up [18]. By a considerably simpler procedure, yielding 58% of nitro-compound **4**, the pyridine is reacted with DNP in $MeNO_2$. The reaction mixture is poured into a solution of $NaHSO_3$ in MeOH-H_2O (3:1) before product isolation [18]. By minor adjustments of the latter procedure, mainly by increasing the reaction time and by precooling the $NaHSO_3$-MeOH/H_2O solution, we were able to increase the yield of 4-acetyl-3-nitropyridine (**4**) from 58 to 73%, comparable to the former SO_2-H_2O procedure. The nitropyridine **4** was reduced with sodium hyposulfite [19] to prevent reduction of the ketone. Amine **5** was obtained in 69% yield, as reported in the literature [20]. Reduction with H_2/Pd or $Pd(OAc)_2/Et_3SiH$ gave considerably lower yields (32–39%).

The preparation of azacinnoline **2** was based on the Borsche approach [21] for the synthesis of cinnolin-4(1*H*)-one (**IVb**) from 2-aminoacetophenone. Diazotization of amine **5** with HCl/$NaNO_2$ in EtOH and subsequent cyclization in alkalic medium by addition of NaOH in EtOH/H_2O afforded the azo-coupling product **2** (38%). Several attempts to optimize the reaction conditions by applying different alkalic systems for the cyclization, such as NaOH (aq), $NaHCO_3$ (aq, sat), phosphate buffer (pH 7.5), and Et_3N, afforded lower yield of product **2**. The following conditions were critical to obtain successful cyclisation. Initially, when applying standard diazotization conditions ($NaNO_2$/HCl/H_2O), we were only able to isolate low yields (<10%) of product **2**. The yield increased significantly by using EtOH as a solvent for the diazotization. Cyclization was favored at pH 7–10. The temperature should strictly be kept below 0°C while adding the basic solution to avoid formation of the 4-acetylpyridine reduction product **3**. It was essential to treat the solution of diazonium salt **1** with the basic solution for successful cyclization, as the opposite treatment only afforded traces amounts of product **2**. Ether extraction at pH 12 removed traces of amine **5**

Scheme 2



Reagents and conditions: (i) 1. N_2O_5 in $MeNO_2$, 0 °C, 2. $NaHSO_3$ in MeOH/H_2O; (ii) $Na_2S_2O_4$ in EtOH, reflux, 6 h; (iii) 1. $NaNO_2$ in HCl/H_2O/EtOH, 0 °C, 2. NaOH in H_2O/EtOH, -10 °C; (iv) 1. $NaNO_2$ in p-TsOH/H_2O, 2. KI

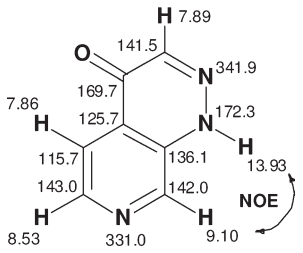


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Figure 1. ^{1}H, ^{13}C, and ^{15}N NMR spectroscopic data of pyridopyridazin-4-one **2** (DMSO-d_6).

and by-product **3**, leaving product **2** in the aqueous phase. The product was afforded by repeated extraction with EtOAc at pH 4–8. Complete product extraction was controlled by TLC monitoring.

A recent report on the iodination of aryl amines in a water-paste form via stable aryl diazonium tosylates [22] demonstrates a simple and effective procedure for the preparation of various aryl iodides. This diazotization method was successfully tested for preparing diazonium ion intermediate **1** to produce 4-acetyl-3-iodopyridine (**6**) from amino-pyridine **5** in high yield (72%). Unfortunately, this method was less successful when applied for the presently studied intramolecular C-azo-coupling of diazonium ion **1**. Only 20% yield of cyclization product **2** was obtained, using a phosphate buffer (pH 7.5) at 0°C for the cyclisation of aryl diazonium tosylate (**1**).

Prototropic tautomerism of heteroaromatic compounds has been studied for decades and is of great biological interest, due to the importance of hydrogen bondings in biological systems [23]. A mixture of compounds **2** and **2′** (Scheme 2) would represent a ketone–phenol tautomeric equilibrium. In general, for simple phenols, the equilibrium lies to the side of phenol to retain the aromatic system. It is, however, well known that the keto form predominates and may be the only detectable form in heterocyclic systems in solution.

NMR spectroscopic investigations revealed that the aza-cinnoline product exists as the keto tautomeric form **2** in DMSO-d_6, in accordance with similar results reported for the benzo-fused cinnoline **IVb** [16]. In general, all NMR data, including results obtained by detailed 2D NMR correlation experiments, ATP, NOESY, HSQC, and HMBC, were in agreement with the NH-keto structure **2**. The NMR signals (δ_H, δ_C, and δ_N) were assigned based on the 2D experiments (Fig. 1). δ_N-Values, reported downfield from liquid ammonia, were obtained from ^{1}H-^{15}N HMBC experiments.

The ^{13}C NMR spectrum of compound **2** showed a characteristic carbonyl signal at 169.7 ppm, in accordance with C4 in the keto-structure **2**. A potential =C—OH signal from the phenolic tautomer **2′** would be expected to appear at higher field. NOESY experiments demonstrated that the acidic proton at 13.93 ppm, represented the =N—NH moiety in compound **2** and not a potential phenolic OH from **2′**, since a through-space proximity between the acidic H1 (13.93 ppm) and H8 (9.10 ppm) was observed. The structure was unambiguously confirmed by heteronuclear multiple bond correlation (HMBC) experiments. The δ_N-values were assigned by heteronuclear long-range correlation ($^{2}J_{N\text{-}H}$ and $^{3}J_{N\text{-}H}$) both between N1 (172.3 ppm) and protons H3, H8; between N2 (341.9 ppm) and proton H3; and between N7 (331.0 ppm) and protons H5, H6, H8. These results left no doubt concerning the keto tautomeric form **2** of the aza-cinnoline product. In particular, the presence of the =N—NH— structure moiety of **2** was confirmed by the essentially different shift values of N1 and N2. The phenol tautomeric form **2′** would have more similar chemical shifts for N1 and N2, due to the presence of a N=N double bond. The chemical shift values for N1 and N2 are in accordance with the reported ^{15}N chemical shifts for cinnolin-4(1*H*)-one (**IVb**) [16]. $CDCl_3$ was not suitable as solvent, as 7-azacinnoline **2** was nearly insoluble in nonpolar solvents. Similar ^{1}H and ^{13}C NMR chemical shift values were observed for azacinnoline **2** in polar protic solvents, such as D_2O and CD_3OD, as in DMSO-d_6. The presence of the carbonyl signal for C4 in CD_3OD (174.0 ppm) and in D_2O (174.6 ppm), confirmed that the keto tautomeric form **2** exclusively dominates in these solvents as well.

In conclusion, pyrido[3,4-c]pyridazin-4(1*H*)-one (**2**) has successfully been prepared by an intramolecular C-azo-coupling, to the best of our knowledge, for the first time. Nitration of 4-acetylpyridine (**3**), reduction, and subsequent diazotization afforded the cyclic azacinnoline product **2**. Based on detailed 2D NMR studies, it was concluded that compound **2** exclusively exists in the NH-keto tautomeric form in DMSO-d_6, CD_3OD, and D_2O.

EXPERIMENTAL

General. Solvents: *pro analysi* quality. NMR: Bruker Avance DPX 400 MHz and Bruker DRX 600 MHz spectrometers. ^{1}H and ^{13}C NMR chemical shift values are reported in ppm downfield from TMS for samples in DMSO-d_6 or $CDCl_3$ and downfield from 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) for samples in CD_3OD or D_2O. *J* values are given in Hz. ^{15}N chemical shifts were referenced indirectly to TMS, using absolute frequency ratio, and are reported in ppm downfield from liquid ammonia [24]. ESI-HRMS accurate mass determination was performed on a Waters QTOF II instrument. IR: Nicolet 20SXC FT-IR spectrophotometer equipped with a Smart Endurance reflexion cell. All melting points are uncorrected and were recorded on a Stuart

apparatus. Flash column chromatography; SiO_2 SDS, 60 Å, 40–63 μm.

Pyrido[3,4-c]pyridazin-4(1*H*)-one (2). To a solution of **5** (106 mg, 0.779 mmol) in ethanol (4 mL) and HCl (conc, 2 mL) at 0°C, an ice-cold solution of $NaNO_2$ (70 mg, 1.01 mmol) in water (1 mL) was added dropwise within 20 min. The reaction mixture was stirred for 1 h and cooled to −10°C. From an ice-cold solution of NaOH (1.08 g, 27 mmol) in H_2O (5 mL)/EtOH (10 mL), the required amount (~24 mmol NaOH) was added drop-wise over 1 h to give pH 8. The reaction was kept stirring for 2 h at −10°C, keeping pH 8–10 by adding additional NaOH/H_2O/EtOH solution. The remaining NaOH/EtOH/H_2O solution was added to give pH 14, and the reaction was allowed to heat to room temperature before water (15 mL) was added. The aqueous solution was washed with ether (10 mL) and acidified with HCl (1*M*) to give pH 4–6. Extraction with EtOAc (6 × 20 mL), drying over Na_2SO_4, evaporation of solvent and flash chromatography (gradient: 5–10% MeOH/CH_2Cl_2) afforded 43 mg (38%) of the title compound **2** as a light orange solid, mp 263–264°C, pure by NMR: R_f 0.37 (10% MeOH/CH_2Cl_2); IR: 2827, 1601, 1561, 1445, 1314, 1081, 881, 853 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ_H 13.93 (br s, 1H, NH), 9.10 (d, J = 0.8 Hz, 1H, H8), 8.53 (d, J = 5.6 Hz, 1H, H6), 7.89 (s, 1H, H3), 7.86 (dd, J = 5.6, 0.8 Hz, 1H, H5); ^1H NMR (400 MHz, CD_3OD): δ_H 9.08 (s, 1H, H8), 8.52 (d, J = 5.2 Hz, 1H, H6), 7.97 (dd, J = 5.2, 1.2 Hz, 1H, H5), 7.92 (s, 1H, H3); ^1H NMR (400 MHz, D_2O): δ_H 9.16 (s, 1H, H8), 8.53 (d, J = 6.0 Hz, 1H, H6), 8.05 (s, 1H, H3), 7.95 (dd, J = 6.0, 0.8 Hz, 1H, H5); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 169.7 (C=O), 143.0 (py-C6), 142.0 (py-C2), 141.5 (CH=N), 136.1 (py-C3), 125.7 (py-C4), 115.7 (py-C5); ^{13}C NMR (100 MHz, CD_3OD): δ_C 174.0, 145.8, 144.8, 144.6, 139.8, 129.2, 119.2; ^{13}C NMR (100 MHz, D_2O): δ_C 174.6, 145.4, 145.2, 143.9, 139.2, 128.8, 118.9; ^{15}N chemical shifts were obtained from ^1H-^{15}N HMBC experiments (600 MHz, DMSO-d_6): δ_N 341.9 (N2), 331.0 (N7), 172.3 (N1); NMR assignments are based on HMBC, HSQC, and NOESY experiments; ESI-HRMS: calcd for $[M+H]^+$ $C_7H_6N_3O$: 148.0505; obsd 148.0505; calcd for $[M+Na]^+$ $C_7H_5N_3NaO$: 170.0325; obsd 170.0321.

4-Acetyl-3-nitropyridine (4). Nitropyridine **4** was prepared from 4-acetylpyridine (**3**) as described elsewhere [18], except for minor modifications. Dinitrogen pentoxide (DNP) was prepared from dinitrogen tetroxide and ozone [25]. DNP (10.0 g, 92.6 mmol) was kept at −78°C and $MeNO_2$ (100 mL) was added. The solution was placed on an ice bath, and acetylpyridine **3** (5.60 g, 46.2 mmol) was added drop-wise over 10 min while stirring. The reaction was stirred for 20 min at 0°C before an ice-cold solution of $NaHSO_3$ (14.5 g, 139 mmol) in H_2O (100 mL)/MeOH (300 mL) was added. The reaction was allowed to heat to room temperature and kept stirring overnight. MeOH was removed under reduced pressure, H_2O (50 mL) was added, and the aqueous solution was extracted with CH_2Cl_2 (3 × 80 mL). The combined organic extracts were washed with HCl (1*M*, 50 mL), $NaHCO_3$ (sat, 100 mL), and water (50 mL), dried over Na_2SO_4, and concentrated under reduced pressure to yield 5.64 g (73%) of the title compound **4** as a slightly yellow solid, pure by NMR.

3-Amino-4-acetylpyridine (5). The title compound **5** was prepared as described in literature [20] from nitropyridine **4** and $Na_2S_2O_4$ in EtOH to afford 69% yield, pure by ^1H NMR; mp 91–92°C (lit. [20], 89–91°C).

4-Acetyl-3-iodopyridine (6). The title compound **6** was prepared by a method described in literature [22]. In a mortar, to amine **5** (100 mg, 0.734 mmol) and water (150 μL) was added *p*-TsOH·H_2O (560 mg, 2.94 mmol). The mixture was ground for 2 min before $NaNO_2$ (152 mg, 2.20 mmol) was added in two portions. The reaction was ground regularly over 10 min with a pestle until TLC showed full conversion of the amine. KI (366 mg, 2.20 mmol) was added, and the grinding was continued for 5 min before minor amounts of water (10 × 100 μL) was added during the next 10 min. Water (3 mL) and then Na_2SO_3 (10%, 10 mL) were added before extraction with EtOAc (3 × 20 mL). The combined extracts were dried over Na_2SO_4 and concentrated under reduced pressure to give 140 mg of brown oil. The crude product was purified by flash chromatography (EtOAc/pentane (1:1)) to give 130 mg (72%) of the title compound **6**, as an yellow oil, pure by NMR; R_f 0.40 (EtOAc/pentane (1:1)); IR 1700, 1393, 1356, 1249, 1010, 829, 680, 604, 590 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ_H 9.03 (s, 1H, py-H2), 8.63 (d, J = 4.8 Hz, 1H, py-H6), 7.32 (d, J = 4.8 Hz, 1H, py-H5), 2.62 (s, 3H, CH_3); ^{13}C NMR (100MHz, $CDCl_3$): δ_C 200.5 (C=O), 158.9 (py-C2), 151.0 (py-C4), 149.3 (py-C6), 121.9 (py-C5), 89.4 (py-C3), 29.4 ($\underline{C}H_3$); NMR assignments are based on HMBC and HSQC experiments; ESI-HRMS: calcd for $[M+H]^+$ C_7H_7INO: 247.9567; obsd 247.9581.

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