A Convenient Approach to the Canthin-4-one Ring System: Total Synthesis of the Alkaloids Tuboflavine and Norisotuboflavine

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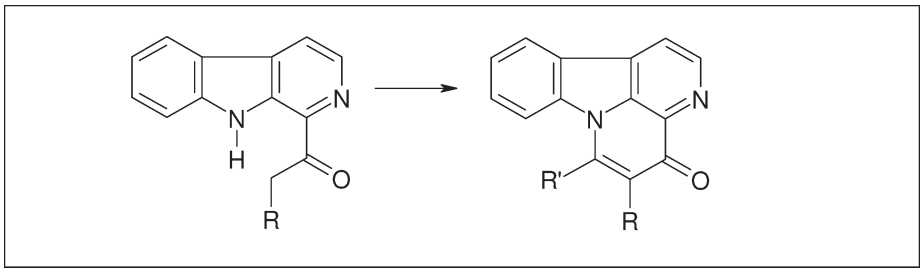

Canthin-4-one, as well as 5- and 6-alkylcanthin-4-ones are readily available through reaction of 1-acyl-β-carbolines with Bredereck's reagent or dimethylacetamide acetals in anhydrous DMF. The intermediate enaminoketones readily undergo cyclization to the canthin-4-ones. The alkaloids tuboflavine and norisotuboflavine were prepared following this methodology.

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INTRODUCTION

Considerable time ago, we published [1] the first total synthesis of the alkaloid annomontine (**3**), a aminopyrimidyl β-carboline from *Annona montana* [2] and *Annona foetida*, showing antileishmanial activity [3]. Readily available 1-acetyl-β-carboline (**1**) [1,4] was reacted with Bredereck's reagent (*tert*-butoxy-bis(dimethylamino)methane) in DMF to give a postulated enaminoketone **2**, which was, without further purification and characterization, converted to the alkaloid **3** by heating with guanidinium carbonate. To our surprise, construction of the aminopyrimidine ring was only possible with guanidinium carbonate under drastic conditions. Free guanidine and other guanidinium salts (hydrochloride, thiocyanate) did not give the desired product. This was in contrast to reports in literature describing an easy conversion of enaminoketones to aminopyrimidines with guanidine or various guanidinium salts [5,6].

RESULTS AND DISCUSSION

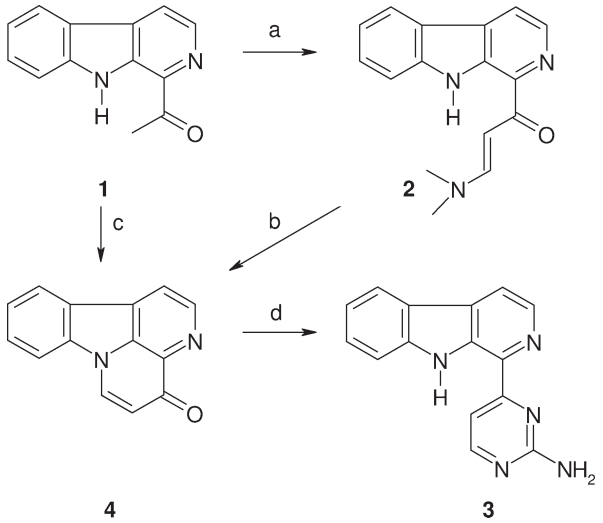
In a reinvestigation of the synthesis described above we intended to isolate the putative intermediate **2**. To our surprise we found that the progress of the reaction strongly depends on the water content of the solvent DMF. Reaction of **1** with Bredereck's reagent in commercially available DMF (water content about 1500 ppm) gave the expected enaminoketone **2**. In contrast, reaction in anhydrous DMF (water content about 50 ppm, as determined by Karl-Fischer titration) gave another product which was unambiguously identified as canthin-4-one (**4**) on the basis of spectroscopic and literature data [7]. Obviously, this product is formed by nucleophilic attack of the indole nitrogen at the enaminoketone moiety, followed by elimination of dimethylamine. In fact, heating of pure enaminoketone **2** in anhydrous DMF in the presence of a catalytic amount of potassium *tert*-butoxide resulted in a clean conversion to canthin-4-one (**4**), whereas heating of **2** in DMF without base for a prolonged time gave only traces of **4**. In the original reaction leading to **4**, the catalytic amount of alkoxide essential for the cyclization is obviously provided by heterolytic cleavage of Bredereck's reagent [8]. So the presence of alkoxide is essential for the cyclization of **2** to canthin-4-one (**4**), obviously by enhancing the nucleophilicity of the indole nitrogen by deprotonation. In wet DMF the alkoxide generated from Bredereck's reagent is likely to be hydrolyzed by water.

Canthin-4-one (**4**) could easily be converted to annomontine (**3**) by heating with guanidinium carbonate in DMF. This ring transformation is obviously initiated by nucleophilic attack of guanidine at the 6-position to affect ring opening, followed by cyclization to the aminopyrimidine ring system (Scheme 1).

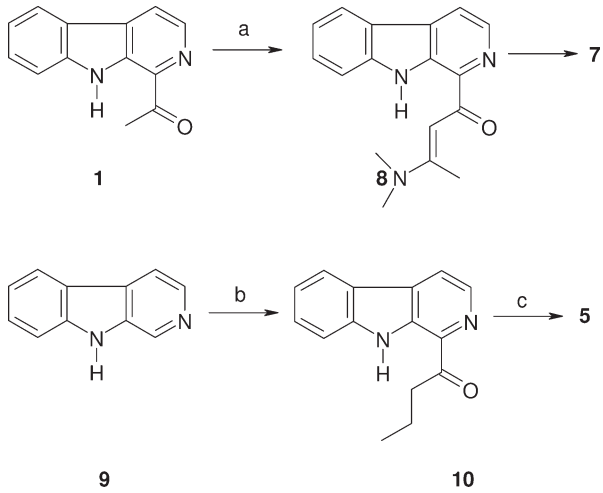
So we found, by chance, a new entry to the canthin-4-one ring system.

Canthin-4-ones represent a small class of three alkaloids isolated from *Pleiocarpa* species (Apocynaceae). Tuboflavine (**5**), isotuboflavine (**6**), and nor-

Scheme 1. Reagents: (a) Bredereck's reagent, wet DMF, reflux; (b) *tert*.-BuOK (cat.), anhydrous DMF, reflux; (c) Bredereck's reagent, anhydrous DMF, reflux; (d) guanidinium carbonate, DMF, reflux.



Scheme 2. Reagents: (a) *N,N*-dimethylacetamide dimethylacetal, anhydrous DMF, 130°C; (b) butyraldehyde, $H_2O/AcOH/H_2SO_4$, then $FeSO_4$, *tert*.-BuOOH/H_2O; (c) Bredereck's reagent, anhydrous DMF, reflux.



isotuboflavine (**7**) were isolated from *Pleiocarpa mutica* [9], **5** was also found in *Pleiocarpa tubicina* [10] (Fig. 1).

Up to now several multi-step syntheses of the alkaloids tuboflavine [11], isotuboflavine [7], norisotuboflavine [7,12], and of unsubstituted canthin-4-one [7] have been published, but none of these offer overall yields higher than 1%. So these total syntheses could only serve as proofs for the structures of the alkaloids, but do not represent efficient approaches to the canthin-4-one ring system.

In order to elucidate the scope of this new anellation protocol, we worked out total syntheses of the alkaloids norisotuboflavine (**7**) and tuboflavine (**5**) following the strategy described above.

Condensation of 1-acetyl-β-carboline (**1**) with *N,N*-dimethylacetamide dimethylacetal in refluxing anhydrous DMF gave, presumably via an intermediate enamine **8**, the alkaloid norisotuboflavine (**7**) in 52% yield.

For the synthesis of tuboflavine (**5**) we prepared the starting material 1-butanoyl-β-carboline (**10**) by *Minisci*–type acylation [13] of norharmane (**9**) with butyraldehyde and *tert*-butyl hydroperoxide/$FeSO_4$ in acidic medium. As we had demonstrated earlier [4], homolytic acylation occurs in a regioselective manner at C-1 with β-carbolines. Diacylated products could only be detected in traces by mass spectroscopy. Condensation of **10** with Bredereck's reagent in refluxing anhydrous DMF readily gave the expected alkaloid **5** in 74% yield (Scheme 2).

In a preliminary screening for antimicrobial activities canthin-4-one (**4**) and norisotuboflavine (**7**) showed strong antibacterial and antifungal activities, whereas tuboflavine (**5**) was inactive.

In conclusion, we have worked out a simple and efficient approach to canthin-4-one as well as 5- and 6-alkylated derivatives thereof, starting from readily available 1-acyl-β-carbolines. Work on further modifications of the canthin-4-one ring system is in progress.

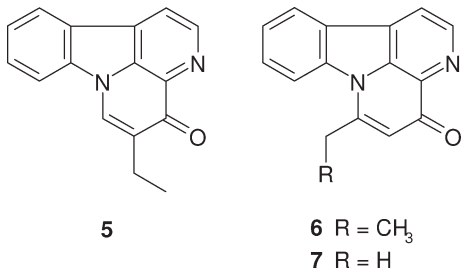


Figure 1. Canthin-4-one alkaloids from *Pleiocarpa* species: tuboflavine (**5**), isotuboflavine (**6**), norisotuboflavine (**7**).

EXPERIMENTAL

General. Elemental analyses were performed on a Carlo Erba CHNO Elemental Analyser. FTIR spectra were recorded as KBr discs on a Pye-Unicam PU-9800 spectrometer, mass spectra on a Finnigan MAT-8430 spectrometer; nmr spectra were recorded with tetramethylsilane as internal standard on a Bruker AM-400 (400.1 MHz ^{1}H, 100.5 MHz ^{13}C) spectrometer, *J* values are given in Hz. Flash column chromatography was carried out on Merck Kieselgel 60 (230–400 mesh).

Commercially available reagent grade DMF (dimethylformamide) was purified by distillation; ''anhydrous'' DMF (water content about 50 ppm) was prepared by distillation followed by storage over activated molecular sieves 4 Å. The water content was determined by Karl-Fischer titration using a Mitsubishi MCI Moisturemeter, model CA-05.

1-(3-Dimethylamino)prop-2-enoyl-β-carboline (2). *tert*-Butoxy-bis(dimethylamino)methane [166 mg (0.95 mmole)] was added to a solution of 105 mg (0.50 mmole) 1-acetyl-β-carboline (**1**) in 8 mL DMF (water content 1580 ppm, equivalent to 0.7 mmole H_2O). The mixture was refluxed under nitrogen for 1 h. After evaporation the residue was purified by flash column chromatography (CH_2Cl_2:ethanol, 14:1, v/v) to give 89 mg (67%) **2** as a brown oil that crystallized overnight, mp 178°C; IR: 3422, 1638, 1625, 1537, 1412, 1243, 1101 cm^{-1}; ^1H NMR (deuteriochloroform) δ 10.82 (s, 1H, NH), 8.49 (d, 1H, J = 5.1 Hz, 3-H), 8.13 (d, 1H, J = 8.7 Hz, 5-H), 8.06 (d, 1H, J = 5.0 Hz, 4-H), 7.96 (d, 1H, J = 12.7 Hz, 3'-H), 7.55 (m, 2H, 7-H, 8-H), 7.27 (m, 1H, 6-H), 6.74 (d, 1H, J = 12.5 Hz, 2'-H), 3.19 (s, 3H, N-CH_3), 3.04 (s, 3H, N-CH_3); ^{13}C nmr (deuteriochloroform) δ 189.3 (C=O), 154.1 (C-3'), 140.9 (C-8a), 138.2 (C-4b), 137.5 (C-3), 135.9 (C-9a), 130.9 (C-4a), 128.6 (C-7), 121.6 (C-5), 120.7 (C-1), 119.9 (C-6), 117.3 (C-4), 111.8 (C-8), 91.5 (C-2'), 45.2 (CH_3), 37.5 (CH_3); ms (EI) m/z 265 (M^+, 17), 235 (15), 222 (48), 221 (100), 220 (19), 182 (19), 98 (26). Hr-ms: Calcd. for $C_{16}H_{15}N_3O$: 265.1215, found: 265.1210.

Canthin-4-one (4) from 1-acetyl-β-carboline (1). Under nitrogen 214 mg (1.23 mmol) *tert*-butoxy-bis-(dimethylamino) methane were added dropwise to a stirred solution of 200 mg (0.95 mmol) (**1**) [1,4] in 20 mL anhydrous DMF (water content about 55 ppm). The mixture was refluxed under nitrogen for 3 h. The solvent was evaporated under reduced pressure and the residue was dissolved in 60 mL ethyl acetate. The organic layer was washed with water (40 mL) and dried with Na_2SO_4. After evaporation the residue was purified by flash column chromatography (CH_2Cl_2:ethanol, 14:1, v/v) to give 156 mg (74%) **4** as a yellow solid. Recrystallization from CH_2Cl_2- heptane gave yellow needles, mp 264°C (lit. [7]: mp (diethyl ether) 270°C); IR: 1645, 1615, 1554, 1506, 1438, 1294, 1223, 1193 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.98 (d, 1H, J =4.6 Hz, 2-H), 8.97 (d, 1H, J = 7.7 Hz, 6-H), 8.47 (d, 1H, J = 4.8 Hz, 1-H), 8.38 (d, 1H, J = 7.7 Hz, 11-H), 8.18 (d, 1H, J = 8.2 Hz, 8-H), 7.79 (dd, 1H, J = 7.3, 8.4 Hz, 9-H), 7.53 (dd, 1H, J = 7.7, 7.3 Hz, 10-H), 6.52 (d, 1H, J = 7.7 Hz, 5-H); ^{13}C NMR (DMSO-d_6) δ 178.7 (C=O), 147.2 (C-2), 139.4 (C-7a), 138.9 (C-3a), 135.0 (C-11c), 134.3 (C-6), 133.7 (C-11b), 131.5 (C-9), 125.1 (C-10), 124.6 (C-11), 124.3 (C-11a), 119.9 (C-1), 117.1 (C-5), 112.7 (C-8); ms (EI) m/z 220 (M^+, 100), 192 (28), 165 (12), 139 (8); Anal. Calcd. for $C_{14}H_8N_2O$: C, 76.35; H, 3.66; N, 12.72. Found: C, 76.02; H, 3.27; N, 12.48.

Canthin-4-one (4) from enaminoketone 2. A catalytic amount (ca. 3 mg) of potassium *tert*-butoxide was added to a refluxing solution of 89 mg (0.34 mmol) **2** in 5 mL anhydrous DMF under nitrogen. Refluxing was continued for 4 h. After cooling to room temperature 20 mL 10% $NaHCO_3$-solution were added and the mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed 3 times with water and dried with $MgSO_4$. After evaporation 59 mg (80%) of pure canthin-4-one (**4**) was obtained.

Annomontine (3). Canthin-4-one (**4**) [100 mg (0.45 mmol)] was dissolved in 10 mL DMF, then 244 mg (1.35 mmol) guanidinium carbonate was added and the mixture was refluxed under nitrogen for 7 h. The solvent was evaporated under reduced pressure and the residue was dissolved in 40 mL ethyl acetate. The organic layer was washed with 30 mL water and dried with Na_2SO_4. After evaporation the residue was purified by flash column chromatography (CH_2Cl_2:ethanol, 14:1, v/v) to give 70 mg (59%) **3** as a yellow solid. The product was identified by comparison with an authentic sample. The spectroscopic data were in full accordance with those described in lit. [1].

Norisotuboflavine (7). Under nitrogen 231 mg (1.74 mmol) *N*,*N*-dimethylacetamide dimethylacetal were added dropwise to a stirred solution of 200 mg (0.95 mmol) 1-acetyl-β-carboline (**1**) in 20 mL anhydrous DMF. The mixture was heated under nitrogen at 130°C for 24 h. The solvent was evaporated under reduced pressure and the residue was dissolved in 60 mL ethyl acetate. The organic layer was washed with water (40 mL) and dried with Na_2SO_4. After evaporation the residue was purified by flash column chromatography (CH_2Cl_2:ethanol, 14:1, v/v) to give 115 mg (52%) **7** as a yellow solid. Recrystallization from CH_2Cl_2-heptane gave pale yellow needles, mp 294°C (lit. [7,9,11]: mp (diethyl ether) 298–300°C, (methanol) 282–284°C, (methanol) 294–296°C); IR: 1633, 1607, 1496, 1428, 1289, 1220 cm^{-1}; ^1H NMR (deuteriochloroform) δ 8.92 (d, 1H, J = 4.7 Hz, 2-H), 8.08 (d, 1H, J = 7.7 Hz, 11-H), 7.99 (d, 1H, J = 4.7 Hz, 1-H), 7.83 (d, 1H, J = 8.4 Hz, 8-H), 7.60 (m, 1H, 9-H), 7.42 (dd, 1H, J = 7.5, 7.6 Hz, 10-H), 6.35 (s, 1H, 5-H), 2.87 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 178.8 (C=O), 147.0 (C-6), 146.7 (C-2), 140.1 (C-7a), 138.5 (C-3a), 135.4 (C-11c), 133.4 (C-11b), 130.9 (C-9), 125.4 (C-11a), 124.6 (C-10), 123.7 (C-11), 118.2 (C-1), 117.6 (C-5), 114.4 (C-8), 21.3 (CH_3); ms (EI) m/z 234 (M^+, 100), 217 (15), 195 (25), 168 (62), 139 (22) and 113 (27); Anal. Calcd. for $C_{15}H_{10}N_2O$: C, 76.91; H, 4.30; N, 11.96. Found: C, 77.05; H, 4.00; N, 11.78.

1-Butanoyl-β-carboline (10). Norharmane (**9**) [200 mg (1.19 mmol)] was suspended in an ice-cooled mixture of water (3 mL), glacial acetic acid (3 mL) and conc. sulfuric acid (0.6 mL) by means of ultrasound irradiation. 0.50 g (6.9 mmol) butyraldehyde were added. Then a solution of 1.0 g (3.6 mmol) $FeSO_4 \times 7\ H_2O$ in 3.6 mL water and 0.5 mL (3.6 mmol) *tert*-BuOOH (70% solution in water) were added simultaneously under stirring. After 1 h at 0°C the mixture was poured into water (100 mL), neutralized with solid K_2CO_3 and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were dried with Na_2SO_4 and evaporated. The residue was purified by flash column chromatography (ethyl acetate:hexane, 1:4, v/v) to give 180 mg (64%) **10** as pale yellow crystals, mp 152°C; IR: 3401, 1663, 1490, 1452, 1430, 1317, 1200, 1137 cm^{-1}; ^1H NMR (deuteriochloroform) δ 10.34 (s, 1H, N—H), 8.52 (d, 1H, J = 4.9 Hz, 3-H), 8.14 (d, 1H, J = 7.9 Hz, 5-H), 8.13 (d, 1H, J = 4.9 Hz, 4-H), 7.53 (m, 2H, 7-H, 8-H), 7.32 (m, 1H, 6-H), 3.39 (m, 2H, 2'-H), 1.88 (m, 2H, 3'-H), 1.08 (t, 3H, J = 7.4 Hz, CH_3); ^{13}C NMR (deuteriochloroform) δ 205.4 (C=O), 141.1 (C-8a), 138.1 (C-3), 135.9 (C-9a), 135.5 (C-1), 131.5 (C-4a), 129.2 (C-7), 121.8 (C-5), 120.6 (C-6, C-4b), 118.9 (C-4), 111.9 (C-8), 39.6 (C-2'), 17.8 (C-3'), 14.0 (C-4'); ms (EI) m/z 238 (M^+, 42), 223 (35), 210 (38), 195 (20), 182 (50), 168 (100), 140 (40), and 91 (32); Anal. Calcd. for $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76. Found: C, 75.97; H, 5.68; N, 11.49.

Tuboflavine (5). Under nitrogen 154 mg (0.89 mmol) *tert*-butoxy-bis(dimethylamino)methane was added dropwise to a stirred solution of 100 mg (0.42 mmol) 1-butanoyl-β-carboline (**10**) in 15 mL anhydrous DMF. The mixture was refluxed under nitrogen for 6 h. The solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography (CH_2Cl_2:ethanol, 19:1, v/v) to give 77 mg

(74%) **5** as a yellow solid. Recrystallization from CH_2Cl_2-heptane gave yellow needles, mp 217°C (lit. [10,12]: mp 207–208°C, 216°C (acetone-hexane)); IR: 1610, 1573, 1510, 1470, 1438, 1345, 1292, 1166 cm^{-1}; ^1H NMR (CF_3COOD) δ 9.23 (d, 1H, J = 5.9 Hz, 2-H), 8.90 (d, 1H, J = 5.9 Hz, 1-H), 8.82 (s, 1H, 6-H), 8.51 (d, 1H, J = 8.0 Hz, 11-H), 8.11 (m, 2H, 8-H, 9-H), 7.81 (m, 1H, 10-H), 2.94 (q, 2H, J = 7.5 Hz, 1'-H), 1.45 (t, 3H, J = 7.5 Hz, 2'-H); ^{13}C NMR (CF_3COOD) δ 173.5 (C=O), 144.7 (C-7a), 143.3 (C-3a), 140.5 (C-2), 136.5 (C-6), 134.7 (C-11c), 133.6 (C-11b), 133.5 (C-9), 127.8 (C-10), 126.5 (C-11), 126.4 (C-11a), 122.4 (C-5), 119.9 (C-1), 112.3 (C-8), 20.9 (C-1'), 10.9 (C-2'); ms (EI) m/z 248 (M^+, 100), 247 (99), 192 (25), 151 (30), 91 (24); Anal. Calcd. for $C_{16}H_{12}N_2O$: C, 77.40; H, 4.87; N, 11.28. Found: C, 77.07; H, 4.85; N, 11.27.

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