Bioorganic & Medicinal Chemistry Letters 25 (2015) 3822–3824

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Total synthesis of cordatanine, structural reassignment of drymaritin, and anti-inflammatory activity of synthetic precursors

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ARTICLE INFO

Article history:
Received 22 June 2015
Revised 20 July 2015
Accepted 22 July 2015
Available online 28 July 2015

Keywords:
Cordatanine
Drymaritin
Kumujian A

ABSTRACT

In this study, cordatanine, with a canthin-6-one skeleton, was totally synthesized in four steps via a Pictet–Spengler reaction using tryptamine and methyl glyoxylate with a total yield of 8%. The NMR spectra of synthesized cordatanine compared well with those of drymaritin isolated by Hsieh et al., confirming the need to revise the original structural assignment. In addition, kumujian A, a synthetic intermediate, showed significant anti-inflammatory effects, inhibiting both superoxide anion generation (IC_{50} 4.87 μg/mL) and elastase release (IC_{50} 6.29 μg/mL).

In 2004, Hsieh et al. reported the isolation of a new alkaloid, identified as 5-methoxycanthin-4-one and named drymaritin, from *Drymaria diandra* (Fig. 1).[1] However, in 2009, Wetzel et al. undertook the total synthesis of 5-methoxycanthin-4-one (drymaritin), but the synthetic product showed spectroscopic data significantly different from those of the *Drymaria* alkaloid, which was subsequently proposed to be cordatanine (4-methyoxycanthin -6-one) (Fig. 1).[2] Based on our long-term interest in canthin-6 -one, this reassignment drew our attention. Herein, we synthesized cordatanine unambiguously by a four-step procedure to validate the exact structure.

Alkaloids with an indole backbone, such as carbazole, β-carboline, or canthin-6-one, usually show various bioactivities, including anti-inflammatory, cytotoxic, or anti-HIV effects. Inflammation plays a key role in Alzheimer's disease, diabetes, atherosclerosis, cancers, and other diseases. Thus, we also evaluated the anti-inflammatory effects of cordatanine and its synthetic intermediates.

The total synthesis of canthin-6-one has been studied extensively, but suffered initially from low yields in the aldol condensation used to close the D ring. In 2005, Soriano-Agatón et al. solved

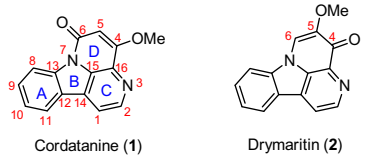

Figure 1. Structures of cordatanine and drymaritin.

this problem by using tryptamine as starting material.[3] Reaction with succinic anhydride afforded the corresponding amide, which was treated with $POCl_3$ under Bischler–Napieralski conditions to produce a key tricyclic imine intermediate. Finally, intramolecular cyclization using diazabicycloundecene (DBU) and concurrent oxidation of the C ring afforded canthin-6-one in high yield. However, this synthetic strategy was not suitable for canthin-6-ones substituted at the C4 or C5 position. Therefore, we decided to follow the procedure of Takasu et al. and adapt a Pictet–Spengler reaction for ring closure (Scheme 1).[4]

Tryptamine was used as starting material and underwent a Pictet–Spengler reaction with ethyl glyoxylate followed by direct oxidative aromatization catalyzed with Pd/C to give kumujian A (**3**) (42%). Aldol condensation using sodium bis(trimethylsilyl)amide (NaHMDS) and ethyl acetate (EA) produced an acetate carbanion, which reacted with **3** to give **4**.[5] The best yield (64%)

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http://dx.doi.org/10.1016/j.bmcl.2015.07.074
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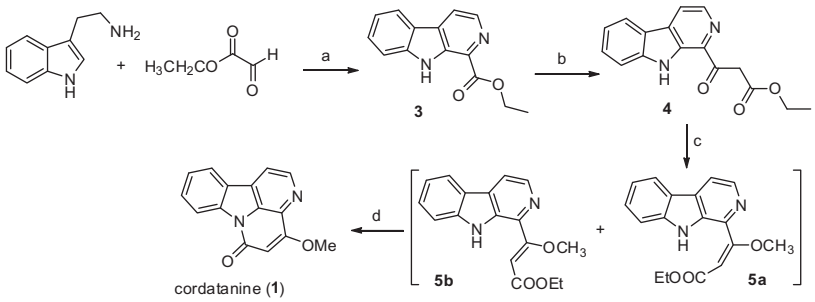

Scheme 1. Synthetic steps to cordatanine. Reagents and conditions: (a) (i) HCl, EtOH, (ii) Pd/C, toluene, reflux; (b) (i) NaHDMS, EtOAc, (ii) **3**, THF, −78 °C; (c) Cs_2CO_3, MeOMs; (d) NaH, THF.

Table 1
Comparison of *O*-methylation conditions

Solvent	Methylation agent	Base or acid	Temperature	Time (h)	**5a**+**5b** yield (%)
DMF	MeOMs	CsF	rt	24	48
DMF	MeOMs	Cs_2CO_3	rt	6	51
DMF	MeOTs	CsF	rt	24	43
DMF	MeOTs	Cs_2CO_3	rt	8	45
Acetone	MeI	K_2CO_3	rt	2	0[a]
Acetone	Me_2SO_4	K_2CO_3	Reflux	6	0[a]
MeOH	$HC(OMe)_3$	H_2SO_4	Reflux	12	0
MeOH	$HC(OMe)_3$	TsOH	Reflux	12	0

[a] Instead of *O*-methylation, α-carbon methylation predominates.

Table 2
Comparison of NMR spectra of cordatanine prepared by total synthesis and drymaritin isolated by Hsieh et al.

Position	Cordatanine	Drymaritin (Hsieh)	Cordatanine	Drymaritin (Hsieh)
	δ_H	δ_H	δ_C	δ_C
1	8.03 (d, *J* = 5.0 Hz)	7.90 (d, *J* = 4.8 Hz)	145.1	144.9
2	8.86 (d, *J* = 5.0 Hz)	8.75 (d, *J* = 4.8 Hz)	117.0	116.8
3	—	—	—	—
4	—	—	161.0	160.8
5	6.22 (s)	6.11 (s)	101.9	101.8
6	—	—	164.2	163.9
7	—	—	—	—
8	8.67 (d, *J* = 7.8 Hz)	8.53 (d, *J* = 8.4 Hz)	117.0	116.9
9	7.73 (d, *J* = 7.8 Hz)	7.63 (d, *J* = 8.4 Hz)	131.0	130.9
10	7.53 (d, *J* = 7.8 Hz)	7.43 (d, *J* = 8.4 Hz)	125.0	125.0
11	8.13 (d, *J* = 7.8 Hz)	7.99 (d, *J* = 8.4 Hz)	122.7	122.5
12			124.6	124.2
13			139.4	139.2
14			130.7	130.5
15			132.1	131.8
16			132.2	131.9
OCH_3	4.15 (s)	4.08 (s)	56.9	56.8

was achieved with a 1:7:5.5 ratio of **3**/NaHMDS/EA. Under basic conditions, removal of an α-proton from the β-keto ester would result a tautomeric enolate anion. In order to obtain compound **5a**+**5b** resulting from *O*-methylation, different reaction conditions, including Cs_2CO_3 or K_2CO_3 as base, as well as different polar solvents and methylation agents, were investigated (Table 1). The results showed that only cesium carbonate or cesium fluoride produced the desired *O*-methylated products. Otherwise, the product from α-carbon methylation predominated. In addition, the combination of methyl methanesulfonate (MeOMs) and cesium carbonate gave the best yield (51%) of **5a**+**5b**.[6] Furthermore, work-up procedure also influenced product distribution. The ratio of **5a** to **5b** was 1:2, if water was added directly to stop the reaction. Removal of DMF in vacuo followed by direct purification led to a 3:1 ratio of **5a**/**5b**. Thus, the content of water influenced the product distribution. Finally, ring closure was achieved by intramolecular nucleophilic substitution using sodium hydride in dilute THF to obtain cordatanine (61%).[7]

Table 3
Comparison of NMR spectra of 'drymaritin' isolated by Hsieh et al. and drymaritin synthesized by Wetzel et al.

Position	Drymaritin		Drymaritin	
	δ_H (Hsieh)	δ_H (Wetzel)	δ_C (Hsieh)	δ_C (Wetzel)
1	7.90 (d, *J* = 4.8 Hz)	8.13 (d, *J* = 4.8 Hz)	144.9	118.4
2	8.75 (d, *J* = 4.8 Hz)	9.05 (d, *J* = 4.8 Hz)	116.8	146.5
3	—	—	—	—
4	—		160.8	173.9
5	—		163.9	149.6
6	6.11 (s)	7.94 (s)	101.8	113.4
7	—		—	—
8	8.53 (d, *J* = 8.4 Hz)	7.73 (m)	116.9	110.6
9	7.63 (d, *J* = 8.4 Hz)	7.74 (m)	130.9	130.9
10	7.43 (d, *J* = 8.4 Hz)	7.49 (m)	125.0	124.1
11	7.99 (d, *J* = 8.4 Hz)	8.17 (d, *J* = 7.8 Hz)	122.5	123.9
12			124.2	124.0
13			139.2	139.5
14			130.5	133.0
15			131.8	132.3
16			131.9	137.0
OCH_3	4.08 (s)	4.03 (s)	56.8	57.4

Proton and carbon NMR spectra of our synthesized cordatanine were compared with those of drymaritin isolated by Hsieh et al. (Table 2). The D ring carbon signals (C4, C5, C6) as well as proton signal of the double bond hydrogen in the isolated drymaritin were quite similar to those of cordatanine synthesized by us, but unlike those of the drymaritin synthesized by Wetzel et al. (Table 3). Accordingly, the compound originally isolated by Hsieh et al. was mistakenly assigned as drymaritin (5-methoxycanthin-4-one) and should be cordatanine (4-methyoxycanthin-6-one). The NMR differences reflect the sequence differences in the D ring linker between the pyrrole B ring and pyridine C ring. For instance, the carbonyl signal occurs at ca. δ_C 164 in cordatanine (C-6), but at much lower field, δ_C 173.9, in drymaritin (C-4). In addition, the proton chemical shift of the alkene hydrogen is much lower in drymaritin (H-6, δ_H 7.94) than in cordatanine (H-5, δ_H 6.11–6.22).

All of the synthetic compounds were screened for anti-inflammatory activity by evaluating their inhibition of superoxide anion generation and elastase release (Table 4). Among all

Table 4
Effects of compounds **1**, **3**, **4**, (**5a**+**5b**) on superoxide anion generation and elastase release in FMLP/CB-induced human neutrophils

Compound	Superoxide anion		Elastase release	
	IC_{50} (μg/mL)	Inhibition (%)	IC_{50} (μg/mL)	Inhibition (%)
1	>10	29.50 ± 2.72	>10	8.20 ± 2.60
3	4.87 ± 0.59	93.92 ± 6.25	6.29 ± 0.22	70.75 ± 3.48
4	>10	10.48 ± 3.94	>10	3.06 ± 1.15
5a+5b	>10	49.53 ± 0.92	>10	26.99 ± 3.71

compounds, compound **3** showed the best activity with inhibitory IC_{50} values of 4.87 and 6.29 μg/mL in the superoxide anion generation and elastase release assays, respectively.

In this study, cordatanine was totally synthesized in four steps via a Pictet–Spengler reaction using tryptamine and methyl glyoxylate with a total yield of 8%. The NMR spectra of synthesized cordatanine were compared with those of drymaritin isolated by Hsieh et al. and the mistaken structural assignment was confirmed. In addition, the synthetic intermediate, kumujian A, showed significant anti-inflammatory effects with IC_{50} values against superoxide anion generation and elastase release of 4.87 and 6.29 μg/mL, respectively.

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5. Ethyl 3-oxo-3-(9*H*-pyrido[3,4-*b*]indol-1-yl)propanoate (**4**). Under nitrogen, ethyl acetate (EA) (0.223 mL, 2.095 mmol) in anhydrous THF (15 mL) was cooled to −78 °C and NaHMDA (2.93 mL, 2.93 mmol) was added slowly. After 30 min, compound **3** was added slowly and the reaction mixture was stirred for 2 h under −78 °C. The reaction was monitored by TLC. Water and ammonium chloride solution was added to stop the reaction. After the reaction mixture returned to room temperature, it was partitioned with EA, washed with brine and dried over $MgSO_4$. The crude product was purified with silica gel column chromatography (*n*-hexane/EA = 6:1–4:1) to afford the desired compound. Yield: 64%. Yellow powder. Mp 139.4–141.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.21 (s, 1H), 8.53 (d, *J* = 5.2 Hz, 1H), 8.16 (d, *J* = 5.2 Hz, 1H), 8.15 (d, *J* = 8.0 Hz, 1H), 7.62 (td, *J* = 8.0, 1.2 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.34 (td, *J* = 8.0, 1.2 Hz, 1H), 4.39 (s, 2H), 4.24 (q, *J* = 7.2 Hz, 2H), 1.27 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.0, 168.2, 141.3, 137.9, 135.6, 134.2, 132.0, 129.6, 121.9, 121.0, 120.4, 119.4, 112.0, 61.2, 44.9, 14.1. HR-ESI-MS: *m*/*z* calcd for $C_{16}H_{15}N_2O_3$ 283.1083, found $[M]^+$ 283.1084.
6. (*E*,*Z*)-Ethyl 3-methoxy-3-(9*H*-pyrido[3,4-*b*]indol-1-yl)acrylate (**5**). To compound **4** (75 mg, 0.265 mmol) in DMF (10 mL), Cs_2CO_3 (129 mg, 0.397 mmol) and methyl methanesulfonate (MeOMs, 0.044 ml, 0.397 mmol) were added under inert atmosphere. The reaction mixture was stirred at ambient temperature for 6 h until starting material disappeared. After DMF was removed in vacuo, water was added and EA was used for partitioning. The organic layer was washed with brine and dried over $MgSO_4$. The crude product underwent silica gel column chromatography (*n*-hexane/EA = 4:1–3:1) to afford the desired compound. Yield: 51%. Yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.22 (s, 1H), 8.44 (d, *J* = 4.8 Hz, 1H), 8.11 (d, *J* = 7.8 Hz, 1H), 7.98 (d, *J* = 4.8 Hz, 1H), 7.52 (t, *J* = 7.8 Hz, 1H), 7.46 (d, *J* = 7.8 Hz, 1H), 7.29 (t, *J* = 7.8 Hz, 1H), 6.67 (s, 1H), 4.25 (q, *J* = 7.2 Hz, 2H), 4.13 (s, 3H), 1.32 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0, 165.4, 140.2, 138.8, 133.8, 130.9, 128.9, 121.5, 120.7, 120.2, 116.0, 111.5, 101.5, 61.6, 59.9, 14.2. HR-ESI-MS: *m*/*z* calcd for $C_{17}H_{17}N_2O_3$ 297.1239, found $[M]^+$ 297.1240.
7. 4-Methoxy-6*H*-indole[3,2,1-*ij*][1,5]naphthyridin-6-one (**1**). Under inert atmosphere, NaH (60%, 10.8 mg, 0.270 mmol) was washed with *n*-hexane and then anhydrous THF (30 mL) was added. The mixture of compounds **5a**+**5b** (40 mg, 0.135 mmol) was added and stirring continued for 16 h. Water was added to stop the reaction and THF was removed in vacuo. $CHCl_3$ and EA were used for partitioning. The organic layer was washed with brine and dried over $MgSO_4$. The crude product was purified by using Al_2O_3 column chromatography (benzene/$CHCl_3$ = 13:1–5:1) to afford cordatanine. Yield: 61%. Yellow powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.86 (d, *J* = 5.0 Hz, 1H), 8.67 (d, *J* = 7.8 Hz, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 8.03 (d, *J* = 5.0 Hz, 1H), 7.73 (t, *J* = 7.8 Hz, 1H), 7.53 (t, *J* = 7.8 Hz, 1H), 6.22 (s, 1H), 4.15 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.2, 161.0, 145.1, 139.4, 132.2, 132.1, 131.0, 130.7, 125.1, 124.5, 122.7, 117.0, 117.0, 101.9, 56.9. HR-ESI-MS: *m*/*z* calcd for $C_{15}H_{11}N_2O_2$ 251.0820, found $[M]^+$ 251.0821.