Revised Structure of the Alkaloid Drymaritin

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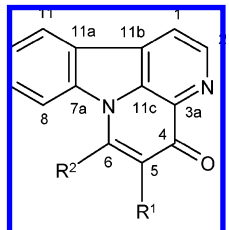
In 2004, a new anti-HIV alkaloid named drymaritin was isolated from *Drymaria diandra*. The authors identified the alkaloid as 5-methoxycanthin-4-one on the basis of spectroscopic data. Here we describe a synthetic approach that unambiguously gave 5-methoxycanthin-4-one, but the synthetic product showed spectroscopic data significantly different from those of the *Drymaria* alkaloid. Extensive re-evaluation of the spectroscopic data published for this and related alkaloids has led to the conclusion that drymaritin does not have a canthin-4-one backbone, but is identical to the known alkaloid cordatanine (4-methoxycanthin-6-one).

In 2004, Hsieh et al.[1] reported on the isolation and identification of a new alkaloid named drymaritin from *Drymaria diandra*. The authors identified this alkaloid as 5-methoxycanthin-4-one (**1**) on the basis of NMR spectroscopic investigations including HMBC experiments. The alkaloid showed interesting anti-HIV activity and thus has been mentioned in a number of review articles (and even more Internet Web sites) dealing with bioactive natural products.[2]

From a chemical point of view, drymaritin appeared to be a new member of the very small class of canthin-4-one alkaloids. For a considerable time this class of polycyclic aromatic alkaloids consisted of only three members: tuboflavine (**2a**), norisotuboflavine (**2b**), and isotuboflavine (**2c**), all of them being 5- or 6-alkylcanthin-4-ones isolated from *Pleiocarpa* species (Apocynaceae).[3] Recently we published a highly efficient synthetic approach to substituted canthin-4-ones (including the alkaloids **2a** and **2b**) starting from appropriate 1-acyl-β-carbolines.[4]



1 $R^1 = OCH_3$, $R^2 = H$
2a $R^1 = C_2H_5$, $R^2 = H$
2b $R^1 = H$, $R^2 = CH_3$
2c $R^1 = H$, $R^2 = C_6H_5$

This prompted us to tackle the first total synthesis of the alkaloid drymaritin. Following our general approach, 5-methoxycanthin-4-one (claimed to be drymaritin) should be accessible starting from 1-(methoxyacetyl)-β-carboline (**3**). Compound **3** is an alkaloid named arenarine A from the Chinese plant *Arenaria kansuensis*, and the first total synthesis of **3** was reported by our group some time ago.[5] Having the precursor **3** in hand, we were able to prepare 5-methoxycanthin-4-one (**1**) in a one-pot reaction. Thus, a solution of **3** in anhydrous DMF was heated with a slight excess of Bredereck's reagent (*tert*-butoxybis(dimethylamino)methane) to give the desired canthin-4-one **1** in almost quantitative yield. As amply discussed in our previous paper,[4] this annulation reaction proceeds via an intermediate enaminoketone formed by condensation of Bredereck's reagent with the acidic methylene group of the 1-acyl-β-carboline, followed by an intramolecular addition/elimination reaction to give the canthin-4-one ring system (Scheme 1).

The correct structure of **1** is evident from the synthetic pathway and was confirmed by ^{1}H, ^{13}C, DEPT, HSQC, and HMBC NMR experiments (Table 1). Furthermore, NOE experiments were performed in order to fully correlate the vinylic proton H-6.

The two- and three-bond HMBC correlations of proton H-6 (marked in bold type in Table 1) to the respective carbon atoms of this condensed ring system play a crucial role in the confirmation of the structure. We found correlations of H-6 to the quaternary carbons C-7a and C-11c, but not to C-3a. The signal for C-3a was proven to be the one at δ 137.0 and was clearly differentiated from the signal of C-11c at δ 132.3 since for C-3a we found a correlation to H-2, but none to H-1, while C-11c showed a correlation to H-1.

Finally, NOE experiments revealed the proximity of vinylic proton H-6 to the aromatic proton H-8. The two-dimensional NOESY spectrum showed a correlation peak for these two protons. In the one-dimensional DPFGSE-NOE spectrum a NOE to H-8 after selectively exciting H-6 with a π-pulse was seen. NOEs from H-6 to the methoxy group appear in both spectra. Hence, the structure of synthetic compound **1** was proven unambiguously.

Surprisingly, the spectroscopic data of synthetic **1** did not at all coincide with those published for the alkaloid drymaritin, suggesting that the structure claimed for this alkaloid was not correct. We attempted to contact the corresponding author (Y.-C. Wu) and one coauthor (K.-H. Lee) of the drymaritin paper to obtain detailed NMR data, but, unfortunately, we received no response. Thus, our discussion of the structure of drymaritin is based solely on our own spectra and on data available from the literature.

On the supposition that at least the molecular formula reported for drymaritin ($C_{15}H_{10}N_2O_2$) is correct, we conducted a database search for constitutional isomers having the same molecular composition and for secondary metabolites that have previously been isolated from *Drymaria* species. The search for "Drymaria alkaloids" gave evidence that canthin-6-ones occur in this plant family. So, Wen-sen[6] isolated 4-methoxycanthin-6-one (**4**; named "cordatanine") from *Drymaria cordata* in 1986, and in 2003 the same alkaloid was isolated from *Drymaria diandra* [sic!] by a Chinese group.[7] Unfortunately, the author of this publication also did not supply analytical data to us upon request.

Taking into consideration that drymaritin might be a canthin-6-one with a molecular formula of $C_{15}H_{10}N_2O_2$, only two structures made sense: the above-mentioned 4-methoxy-canthin-6-one (**4**) and 5-methoxycanthin-6-one (**5**). Compound **5** is also a well-known secondary metabolite from various plants.[8] Unfortunately, the analytical data published for **4** and **5** are mostly of poor quality and fragmentary. Most helpful for our purpose was the publication of Wen-sen,[6] where ^{1}H NMR data of both **4** and **5** as well as the

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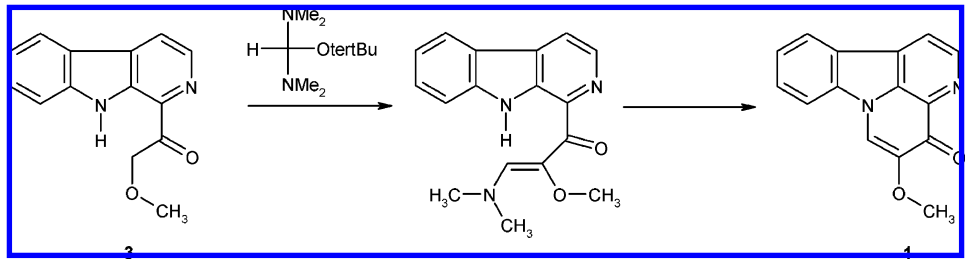
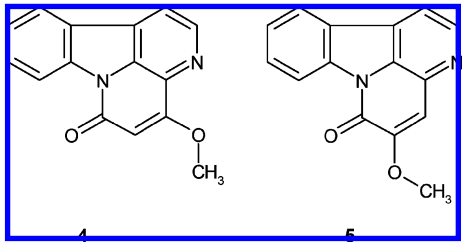
Scheme 1. Synthesis of 5-Methoxycanthin-4-one (**1**)



^{13}C NMR data of **4** are presented. The data reported there for 4-methoxycanthin-6-one (**4**) are in very good accordance with those of the new alkaloid drymaritin, whereas the resonances of the isomer **5** show significant differences. Moreover, we compared the UV data reported for drymaritin with those of 4-methoxycanthin-6-one (**4**)[9] and 5-methoxycanthin-6-one (**5**).[10] We found good accordance with the UV data described by Wen-sen[6] and Scheuer[9b] for 4-methoxycanthin-6-one (**4**); moreover the structure of the sample of **4** used in these investigations was unambiguously confirmed by total synthesis.[9b] Here again, the data reported for 5-methoxycanthin-6-one (**5**) strongly differed from those of drymaritin. Taking all of these findings together, we can assume that the "new" alkaloid drymaritin does not have the proposed structure 5-methoxycanthin-4-one (**1**), but that it is identical to the known canthin-6-one alkaloid cordatanine (**4**).



The erroneous structure reported by Hsieh et al.[1] was obviously based mainly on two outstandingly important HMBC correlations. The first one was the correlation between H-6 and C-11c (assigned as C-15 by Hsieh), and the second one was the correlation between H-6 and carbon C-7a (assigned as C-13 by Hsieh). Both observed cross-peaks should result from ^3J couplings and fit with the proposed structure **1** at first sight. Nevertheless, the structure proposed earlier is not correct.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **1** in $CDCl_3$

position[a]	δ_C[b]	δ_H[b,c]	HMBC[d,e]
1	118.4	8.13 (d, J = 4.8 Hz)	2 (s)
2	146.5	9.05 (d, J = 4.8 Hz)	1 (m)
3			
3a (16)	137.0		2 (m)
4	173.9		**6 (s)**
5	149.6		OCH_3 (s), **6 (w)**
6	113.4	7.94 (s)	
7			
7a (13)	139.5		**6 (w)**, 9 (s), 11 (s)
8	110.6	7.73 (m)	10 (s),
9	130.9	7.74 (m)	10 (w), 11 (s)
10	124.1	7.49 (m)	8 (s)
11	123.9	8.17 (d, J = 7.8 Hz)	
11a (12)	124.0		1 (m)
11b (14)	133.0		2 (s), 11 (m)
11c (15)	132.3		1 (s), **6 (s)**
OCH_3	57.4	4.03 (s)	

[a] In parentheses: deviant numbering used by Hsieh et al.[1] [b] Chemical shifts in ppm relative to TMS. [c] In parentheses: multiplicities; coupling constants J in Hz. [d] HMBC correlations are from proton(s) listed in this column to the indicated carbon atoms. Important correlations of H-6 are marked in bold type. [e] (s) indicating strong, (m) medium, (w) weak.

We take the liberty of discussing how, despite using sophisticated NMR experiments, this misinterpretation might have happened. Considering the first mentioned correlation (H-6 with C-11c, assigned by them as C-15), the authors might have mixed up two ^{13}C NMR signals, since the signal they found for C-3a (assigned by them as C-16; δ 131.9) has almost the same chemical shift as C-11c (assigned by them as C-15; δ 131.8), with a difference in chemical shifts of only $\Delta\delta = 0.1$ ppm. So a correct assignment of the corresponding cross-signal might not have been possible or sufficiently accurate. Another possibility must not be disregarded. Wen-sen[6] reported ^{13}C NMR data for 4-methoxycanthin-6-one (**4**) that are consistent with the data reported for drymaritin, except for carbon C-3a (assigned as C-16 by Hsieh et al.). Hsieh et al. did not find the signal for this carbon at δ 131.9, but at δ 144.8. To concede, this is also a rather crowded region, as another signal (for C-2) appears at δ 144.9. In conclusion. Wen-sen reported on three signals at δ 131.8 and 144.8/144.9, and Hsieh et al. described resonances at δ 131.8/131.9 and 144.9. It is quite tedious speculating whose signal is the artifact.

The second mentioned correlation completing Hsieh's assignment for the position of "H-6" might be a misinterpretation when excluding the possibility for the occurrence of ^4J couplings. Although the authors describe a ^4J coupling tabulated for the HMBC correlation of "C-15" to H-2, this option was not taken into consideration for proton H-6 and carbon C-7a in the structure of isomer **4**. Considering the cross-signal being that for the correlation (^3J) between H-5 and C-3a and allowing for the occurrence of a ^4J coupling between H-5 and C-7a, the structure of drymaritin is now not only in the realm of possibility for that of 4-methoxy-canthin-6-one (**4**) but rather definite.

In addition, there are some expected HMBC correlations for compound **1** clearly evident in our synthetic product but that are totally missing in the tabulation of data for drymaritin: ^3J correlations of C-3a (assigned as C-16 by Hsieh) to H-2, of C-11c (assigned as C-15 by Hsieh) to H-1, and, surprisingly, of H-6 to carbonyl carbon C-4, indicating that the distance between the vinylic proton and the carbonyl group in drymaritin is more likely two bondings, as shown in structure **4**, than three bondings, as in synthetic compound **1**. Admittedly, data derived from a ^{1}H−^{15}N HMBC spectrum (600 MHz) were presented for drymaritin by Hsieh et al.,[1] but on closer inspection none of the observed correlations rule out structure **4**.

In conclusion, we described a total synthesis of 5-methoxycanthin-4-one (**1**), the putative structure of the alkaloid drymaritin. Careful comparison of our spectroscopic data and those found in the literature revealed that drymaritin is not the proposed canthin-4-one **1**, but more likely it is identical to the known canthin-6-one alkaloid cordatanine (**4**). The results presented here demonstrate that structure elucidations based only on spectroscopic data bear some risks of misinterpretation. Once again, our efforts regarding the total synthesis of alkaloids (performed *sine ira et studio*) helped to identify an erroneous structure assignment.[11]

Experimental Section

General Experimental Procedures. Melting points were determined on a Büchi melting point B-540 apparatus and are uncorrected. UV spectra were obtained on a Jasco V-530 UV/Vis spectrophotometer;

IR spectra, on a Perkin-Elmer FT-IR Paragon-1000 spectrometer. NMR spectra were obtained on a Jeol JNMR-GX500 (500 MHz) spectrometer. Mass spectra were recorded on a Hewlett-Packard MS-Engine, electron ionization (EI) 70 eV, chemical ionization (CI) with CH_4 (300 eV). High-resolution EIMS were measured on a jeol JMS GCmate II. Flash column chromatography was performed using silica gel 60 (230−400 mesh, E. Merck, Darmstadt).

5-Methoxycanthin-4-one (1). Arenarine A (**3**) (11.0 mg, 0.046 mmol) was dissolved in 4 mL of anhydrous DMF. Then, under a nitrogen atmosphere and with stirring, 11 mg (0.060 mmol) of *tert*-butoxybis(dimethylamino)methane (Bredereck's reagent) was added. The mixture was refluxed under nitrogen for 4 h; then the solvent was evaporated under reduced pressure and the residue purified by flash column chromatography (silica gel; CH_2Cl_2/ethanol, 14:1, v/v) to give 11.1 mg (97%) of **1** as a yellow, amorphous solid: mp 281 °C (dec); UV (EtOH) λ_{max} (log ε) 216 (4.21), 268 (3.98), 293 (4.04), 325 (3.41), 422 (3.48) nm; IR ν_{max} 3386, 2924, 1608, 1561, 1522, 1451, 1330, 1237, 1093 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 1; CIMS *m*/*z* (rel int) 251 ($[M + 1]^{+\bullet}$, 100), 185 (15), 102 (31); EIMS *m*/*z* (rel int) 250 ($[M]^+$, 100), 221 (28), 192 (64); HREIMS *m*/*z* 250.07397 $[M]^+$ (calcd for $C_{15}H_{10}N_2O_2$, 250.07423).

Supporting Information Available: ^1H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Hsieh, P.-W.; Chang, F.-R.; Lee, K.-H.; Hwang, T.-L.; Chang, S.-M.; Wu, Y.-C. *J. Nat. Prod.* **2004**, *67*, 1175–1177.

(2) (a) Kawasaki, T.; Higuchi, K. *Nat. Prod. Rep.* **2005**, *22*, 761–793. (b) Naithani, R.; Huma, L.; Holland, L. E.; Shukla, D.; McCormick, D. L.; Mehta, R. G.; Moriarty, R. M. *Mini Rev. Med. Chem.* **2008**, *8*, 1106–1133. (c) Cos, P.; Maes, L.; Vlietinck, A.; Pieters, L. *Planta Med.* **2008**, *74*, 1323–1337.

(3) (a) Achenbach, H.; Biemann, K. *J. Am. Chem. Soc.* **1965**, *87*, 4177–4181. (b) Kump, C.; Seibl, J.; Schmid, H. *Helv. Chim. Acta* **1963**, *46*, 498–505.

(4) Puzik, A.; Bracher, F. *J. Heterocycl. Chem.* **2009**, *46*, 770–773.

(5) Bracher, F.; Puzik, A. *J. Heterocycl. Chem.* **2004**, *41*, 173–176.

(6) Wen-sen, C. *Acta Bot. Sin.* **1986**, *28*, 450–452.

(7) Yang, X.-G.; Rong, H.; Bao, Z.-J.; Ding, Z.-T. *Yunnan Daxue Xuebao, Ziran Kexueban* **2003**, *25*, 358–360.

(8) (a) Lassak, E. V.; Polonsky, J.; Jacquemin, H. *Phytochemistry* **1977**, *16*, 1126–1127. (b) Wagner, H.; Nestler, T.; Neszmelyi, A. *Planta Med.* **1979**, *36*, 113–118. (c) Ohmoto, T.; Koike, K. *Chem. Pharm. Bull.* **1983**, *31*, 3198–3204. (d) Cordell, G. A.; Ogura, M.; Farnsworth, N. R. *Lloydia* **1978**, *41*, 166–168.

(9) (a) Nelson, E. R.; Price, J. R. *Austr. J. Sci. Res.* **1952**, *A5*, 768–781. (b) Scheuer, P. J.; Pattabhiraman, T. R. *Lloydia* **1965**, *28*, 95–100.

(10) Nelson, E. R.; Price, J. R. *Austr. J. Sci. Res.* **1952**, *A5*, 563–569.

(11) (a) Bracher, F. *Arch. Pharm. (Weinheim)* **1992**, *325*, 645–648. (b) Bracher, F. *Liebigs Ann. Chem.* **1992**, 1205–1207. (c) Bracher, F.; Hildebrand, D. *Liebigs Ann. Chem.* **1993**, 1335–1337. (d) Ritter, A.; Poschenrieder, H.; Bracher, F. *Z. Naturforsch.* **2009**, *64b*, 427–433.

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