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Establishment of Benzodioxazine Core Structure for Sarcodonin Class of Natural Products by X-ray Analysis

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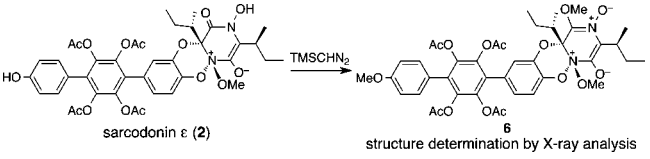
ABSTRACT



Sarcodonin ε (2), isolated from *Sarcodon scabrosus*, was treated with trimethylsilyldiazomethane to yield a crystalline methylated derivative 6. The structure of 6 was determined by X-ray analysis, which confirmed the presence of an unprecedented N(1b)-OMe group, the configuration at N(1b) and the 1,3,4-substituted phenyl ring structure of 2. More importantly, the structures of 6 and 2 have answered the intriguing problem of solving the core structure of the sarcodonin class of natural products, establishing that sarcodonins have a benzodioxazine core structure, rather than the recently proposed benzodioxane aminal core structure.

Since the first isolation of the nitrogenous *p*-terphenyl natural product sarcodonin (**1a**) in 2000,[1] more than 10 sarcodonin classes of natural products have been reported.[2−5] These compounds have been isolated from mushrooms, *Sarcodon leucopus*, *S. scabrosus*, *Hydnellum suaveolens*, *H. geogerirum* and *Phellodon niger* and have been reported to show anti-HIV, antioxidant, and anticancer activity.[2−5] All of these isolation studies have described a characteristic benzodioxazine core structure of sarcodonins as well as an *N*,*N*-dioxide ring junction, which were deduced from 2D-NMR studies and chemical degradation studies leading to a diketopyperazine composed of two isoleucines. In contrast, Baran and co-workers recently reported the possibility of an alternative benzodioxane aminal core structure through synthetic studies of this class of compounds (**1b** for sarcodonin) (Figure 1).[6]

We recently investigated the secondary metabolites of *S. scabrosus*[7] and isolated a sizable amount of a new compound named sarcodonin ε (**2**) that yielded NMR data similar to those of phellodonin[5] (**3**) and a minor substance that was identified as sarcodonin δ[3] (reported structure **4**, revised structure in the present study **5** in Figure 1), obtained from the fruiting bodies of *S. scabrosus*, from which sarcodonin δ was previously isolated. Compounds **2** and **5** yielded an identical acetate (FABMS, *m*/*z* 863 $[M + H]^+$) upon typical acetylation.

The availability of **2** and **5** prompted us to investigate the intriguing structure problem of the sarcodonin class of natural products. In this article we describe evidence in favor of the benzodioxazine core structure and the *N*,*N*-dioxide ring junction.

The structure of sarcodonin ε (molecular formula $C_{39}H_{42}N_2O_{15}$ was deduced from HRFABMS data) was

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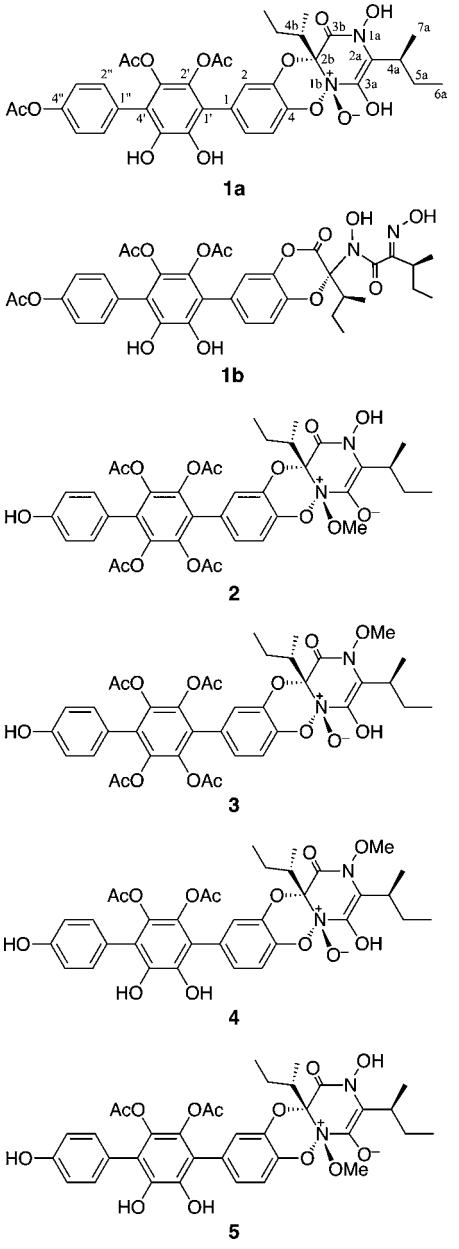


Figure 1. Structures of sarcodonins **1**–**5**.

found to be distinct from phellodonin, as revealed by careful NMR spectral comparison (Table 1), and was thought to be **2** (a most likely tautomeric/resonance structure is depicted) on the basis of the followings. The OMe group was determined to be attached to the nitrogen atom rather than the carbon atom because of the lack of HMBC correlation to any carbon atom. The strongly chelated hydrogen of OH (δ 7.92) showed a clear HMBC correlation to C-2a, but not to C-3a. The MeO signal showed an NOE to H-4b. Compound **2** was not susceptible to epimerization at N(**1b**),[2] suggesting the presence of N(**1b**)-OMe group. Compound **2** formed a C(**3a**)-OAc,C-4″-OAc derivative in good yield, which exhibited ^{13}C data [C-2a (δ 165.6, shifted downfield compared to that (δ 159.1) of **2**) and C-3a (δ 164.2, shifted upfield compared to that (δ 166.4) of **2**)], with both chemical shifts being in agreement with an acylation shift.[8] The MeO signal on N(**1b**) of the acetate also showed an NOE to H-4b. Finally, the methylated compound **7** (vide infra) exhibited NOE correlations between N(**1b**)-OMe and C(**3a**)-OMe and between N(**1b**)-OMe and H-4b. It is of note that the presumed structure of **2** has an unprecedented N(**1b**)-OMe group instead of an N(**1a**)-OMe group, as found in phellodonin (**3**).

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of **2** and **6** (500/125 MHz, in $CDCl_3$)

	2		**6**	
no.	δ_H (mult, J in Hz)	δ_C	δ_H (mult, J in Hz)	δ_C
1	–	128.0	–	127.5
2	6.95 (d, 1.8)	118.5	7.02 (d, 1.8)	118.6
3	–	140.8	–	139.7
4	–	141.6	–	139.1
5	7.13 (d, 8.3)	117.0	7.07 (d, 8.4)	116.5
6	7.02 (dd, 8.3, 1.8)	125.1	6.97 (dd, 8.4, 1.8)	124.6
1′	–	128.8	–	128.9
2′, 6′	–	139.1	–	139.3
3′, 5′	–	139.4	–	139.1
4′	–	130.9	–	130.7
1″	–	123.1	–	123.3
2″,6″	7.16 (d, 8.6)	130.9	7.23 (d, 8.5)	130.7
3″,5″	6.84 (d, 8.6)	115.3	6.92 (d, 8.5)	113.7
4″	–	156.0	–	159.5
2a	–	159.1	–	141.8
3a	–	166.4	–	158.9
4a	3.11 (m)	33.3	3.40 (m)	33.5
5a	1.42 (m), 1.59 (m)	26.8	1.61 (m), 1.96 (m)	24.1
6a	0.87 (t, 7.5)	11.9	0.84 (t, 7.5)	12.4
7a	1.07 (d, 7.1)	15.5	1.24 (d, 7.0)	14.1
2b	–	92.9	–	92.2
3b	–	159.3	–	105.1
4b	2.41 (m)	42.5	2.36 (m)	40.6
5b	1.61 (m), 1.79 (m)	24.6	1.32 (m), 1.96 (m)	23.9
6b	0.99 (t, 7.5)	12.7	0.90 (t, 7.5)	13.0
7b	1.21 (d, 6.9)	13.3	1.10 (d, 6.9)	13.9
2′/6′- OAc	1.99[a] (s)	20.1	1.98[c] (s)	20.1[d]
		168.2[b]		167.3[e]
3′/5′- OAc	1.96[a] (s)	20.1	1.94[c] (s)	20.0[d]
		167.7[b]		167.6[e]
(1b)-OMe	3.95 (s)	67.0	3.71 (s)	64.6
3b-OMe	–	–	3.76 (s)	54.3
4″-OMe	–	–	3.84 (s)	55.2
1a-OH	7.92 (bs)	–	–	–
4″-OH	6.02 (bs)	–	–	–

[a)–d)] Interchangeable within the column.

Generally, the sarcodonin class of compounds is noncrystalline, which has hampered definitive structure determination. Our attempt to obtain a crystalline derivative such as *p*-nitrobenzoyl and camphorsulfonyl esters of **2** was not successful, because the reactions did not proceed cleanly. Fortunately, we have found that treatment of **2** with $TMSCHN_2$ (hexane solution) in MeOH–Et_2O at room temperature overnight under N_2 yielded a crystalline

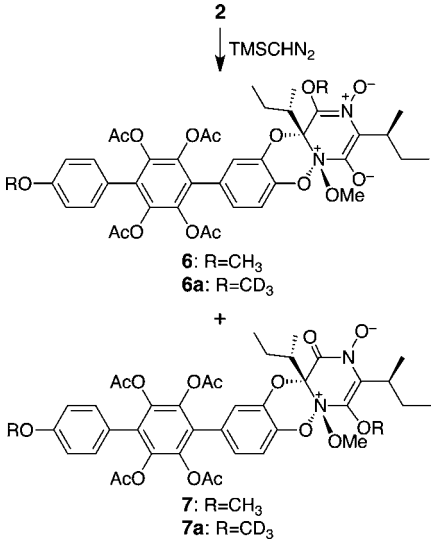
(8) Terui, Y.; Tori, K.; Tsuji, N. *Tetrahedron Lett.* **1976**, 621. Jones, R. A.; Stokes, M. J. *Tetrahedron* **1984**, *40*, 1051.

derivative **6** with 52% yield, together with a minor oily product **7** (m/z 807 $[M + H]^+$) in 23% (Scheme 1).

The ^{1}H NMR and FABMS data (m/z 807 $[M + H]^+$) of **6** indicated that two methyl groups were newly introduced onto **2**. 2D NMR analysis of **6** (Table 1) indicated that a methyl group was located at the C-4″ position and the other methyl group was assigned as C(**3b**)-OMe because both the OMe group (δ 3.76) and H-4b (δ 2.36) had HMBC correlation to C-3b (δ 105.1). It is notable that C-3b (δ 105.1) was significantly shifted upfield compared to that (δ 159.3) of **2**. The ^{1}H and ^{13}C shifts of the MeO group at N(**1b**) were shifted only slightly compared to those of **2** (see Table 1). We were not able to obtain definitive evidence of the existence of the presumed N(**1b**)-OMe linkage in **6**.

Thus, compound **6** was recrystallized from hexane–EtOAc, and a resulting needle (mp 208 °C) was analyzed by X-ray crystallography. The ORTEP drawing is illustrated in Figure 2, which permitted us to determine the structure of **6** including the benzodioxazine core structure. Absolute stereochemistry of the molecule was deduced based on the *S* chirality of the isoleucine-derived moiety.[1] The configuration at N(**1b**) and the structure of the 1,3,4-substituted phenyl ring, previously reported for sarcodonins,[1−5] were substantiated unequivocally for the first time. Furthermore, the unique structure bearing the N(**1b**)-OMe group of **2** was ascertained.

Scheme 1. Methylated Products **6** and **7** Obtained from **2** upon $TMSCHN_2$ Treatment[a]



[a] Compounds **6a** and **7a** were obtained using CD_3OD solvent instead of MeOH.

The observed bond lengths for the core part of **6** in the X-ray structure are as follows: N(**1a**)–C(**2a**) 1.519(9), C(**2a**)–C(**3a**) 1.281(8), C(**3a**)–N(**1b**) 1.519(8), N(**1b**)–C(**2b**) 1.532(8), C(**2b**)–C(**3b**) 1.417(7), C(**3b**)–N(**1a**) 1.357(9), N(**1a**)-O 1.253(7), C(**3a**)–O 1.286(6), and C(**3b**)–O 1.423(7) Å. The bond angles for C(**2b**)–C(**3b**)–N(**1a**), C(**3b**)–N(**1a**)–C(**2a**) and N(**1a**)–C(**2a**)–C(**3a**) were 122.8(6), 117.3(6) and 118.9(6)°, respectively. These data suggested that the iminium *N*-oxide system bears C(**3b**)=N(**1a**)$^+$–O$^-$ and C(**3b**)$^-$–N(**1a**)$^+$=O resonance forms. It appears that the latter form contributes the above-mentioned upfield shift of C-3b (δ 105.1) of **6**.[9]

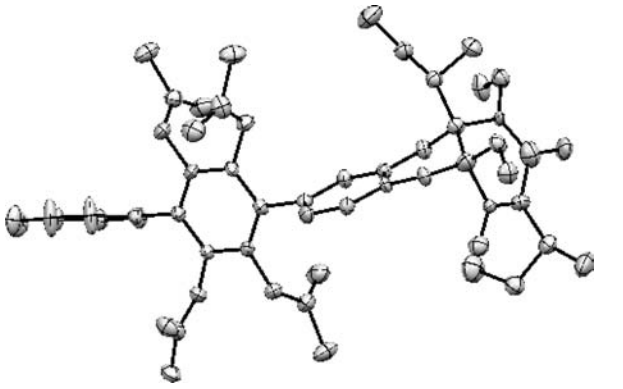


Figure 2. Single-crystal X-ray structure of **6**.

To shed light on the fate of the N(**1b**)-OMe group in **2** during the methylation reaction, **2** was treated with $TMSCHN_2$ in CD_3OD (known to act as a "CD_3" source[10]) instead of MeOH. The ^{1}H NMR analysis of the resulting product **6a** indicated that the OMe group on N(**1b**) was essentially unlabeled (δ_H 3.71), but the OMe group at C(**3b**) was exclusively deuterium labeled (residual CD_2H at δ_H 3.73), as in the OMe group at C-4″. This finding implied that the OMe group at N(**1b**) in **2** remained at the original position and eliminated the possibility of an unexpected migration during the $TMSCHN_2$ treatment. The minor product **7a** had deuterium-labeled OMe groups at C-4″ and C-3a, whereas N(**1b**)-OMe was unlabeled.

Here, we would like to comment on the structure of sarcodonin δ (**5**). Compound **5** should have the same core structure as **2** because both compounds **2** and **5** exhibited essentially identical NMR data for the benzodioxazine core moiety and yielded the same hexa-acetate (acetylated 2′, 3′, 5′, 6′, 4″, C-3a). Furthermore, $TMSCHN_2$ treatment of **5** proceeded in the same manner as that of **2** to yield an oily compound **8** (m/z 751 $[M + H]^+$) as a major product (Scheme 2), which gave essentially identical NMR spectra (N(**1b**)-OMe δ_H 3.71, δ_C 64.7; C(**3b**)-OMe δ_H 3.80, δ_C 54.5) for the core moiety as did **6**, thus confirming the presence of the N(**1b**)-OMe and C(**3b**)-OMe groups in sarcodonin δ. Hence, the structure of sarcodonin δ was revised as **5**, shown in Figure 1.

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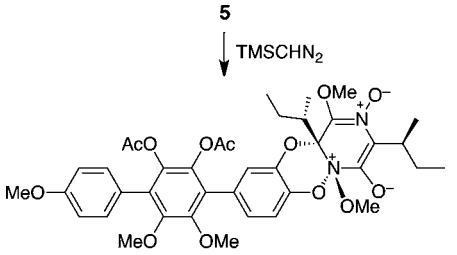
(11) Baran and coworkers have recently completed the synthsis of sarcodonin ε and phellodonin. See the following paper in this issue: Usui, I.; Lin, D. W.; Masuda, T.; Baran, P. S. *Org. Lett.* **2013**, DOI: 10.1021/ol400709f.

Scheme 2. Methylated Product **8** Obtained from **5** upon $TMSCHN_2$ Treatment



In conclusion, X-ray analysis of compound **6**, derived from sarcodonin ε (**2**), established the structures of **6** as well as **2**. The present study has provided conclusive evidence for the core structure of the sarcodonin class compounds. It is concluded that sarcodonins have a benzodioxazine core structure (such as **1a**) as originally reported, but not a benzodioxane aminal core structure.[11]

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Supporting Information Available. Detailed experimental procedures, NMR spectra of compounds **2**, **5**, peracetate of **2**, **6**, **6a**, **7**, **7a** and **8**, and CIF file of **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.