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Absolute Configuration of Norzoanthamine, a Promising Candidate for an Osteoporotic Drug[1]

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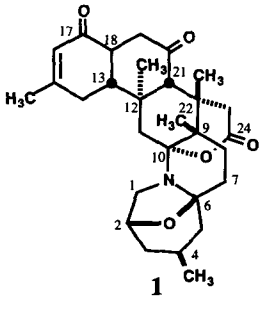
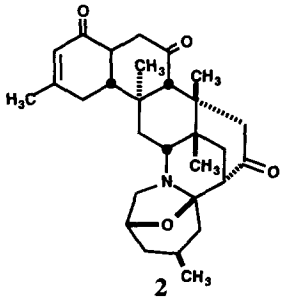
Abstract: The absolute configuration of norzoanthamine (**1**) was determined to be 2*R*, 4*S*, 6*S*, 9*S*, 10*R*, 12*S*, 13*R*, 18*S*, 21*S*, and 22*S* based on ^{1}H NMR spectral data of the MTPA esters of norzoanthamine derivatives. The possible biogenesis of zoanthamines is also proposed.

Norzoanthamine (**1**) is a zoanthamine-type alkaloid from the colonial zoanthid *Zoanthus* sp.[2,3] Norzoanthamines inhibit IL-6 production. Furthermore, norzoanthamine hydrochloride, which suppresses the decrease in bone weight and strength in ovariectomized mice, could be a good candidate for an osteoporotic drug.[4] Although the relative configuration of norzoanthamine was established based on an X-ray crystallographic analysis, the absolute conformation of norzoanthamines, including zoanthamines which have been reported previously, remains unclear. To investigate their biogenesis and mechanism of biological action, we conducted chemical and spectroscopic studies to determine the absolute configuration of norzoanthamines. We report here the absolute stereochemistry of norzoanthamines and a proposed biogenesis.



1



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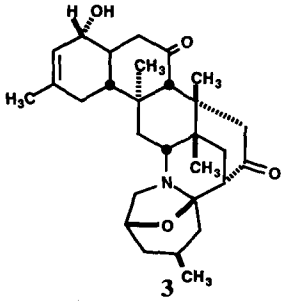

3

Norzoanthamine (**1**) was reduced with $NaBH_4$ to give two derivatives, deoxynorzoanthamine (**2**) and deoxydihydronorzoanthamine (**3**). Interestingly, the ^{13}C NMR spectrum of **2** has revealed the presence of three ketonic carbonyls (δ_C 198.7, 209.0, 211.5), whereas **1** has two ketonic carbonyls [C17 (δ_C 198.4) and C20 (δ_C 209.0)] and one lactone carbonyl with carbon resonances at 172.4 ppm.[2,5] The molecular formula of **2** was assigned to be $C_{29}H_{39}NO_4$ from HREIMS of **2** (*m/z* 465.2889, Δ +1.2 mmu).[6-8] All of the signals were assigned by a detailed comparison of NMR spectral data with those of **1** and by ^{1}H-^{1}H COSY and HMBC

spectra. Carbon networks among C9-C12 were revealed by the observation of crosspeaks in the ^{1}H-^{1}H COSY spectrum (H10/H11a and H10/H11b) and HMBC crosspeaks (28-CH_3/C9, 28-CH_3/C10, 27-CH_3/C11, and 27-CH_3/C12). The HMBC crosspeaks between H10 and C1, and the chemical shift at C10 (δ_C 71.1) suggested connectivity among C1/N and N/C10. Finally, carbon connectivity between C7 and C24 was indicated by ^{1}H-^{1}H COSY crosspeaks (H7 and H8) and HMBC spectra (H7/C6, H7/C24, and H23/C7)(Figure 2). As a result, the planar structure was proposed to be **2**. The relative stereochemistry of **2** was the same as that of **1**, except at C7 and C10.[9] The stereochemistry at C7 and C10 was deduced from the coupling constants of H10 ($J_{10,11a}$= 4.5 Hz and $J_{10,11b}$= 2.2 Hz), NOE between H7 and 28-CH_3, and the mechanism of rearrangement, as shown in Figure 1.

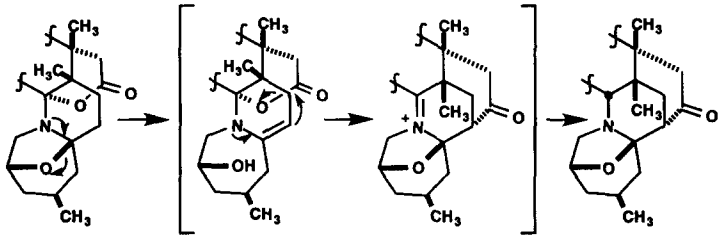

Figure 1. Proposed Mechanism of the Reductive Transformation of **1** into **2**.

The molecular formula of **3** was determined to be $C_{29}H_{41}NO_4$ from HREIMS of **3** (*m/z* 467.3011, Δ -2.1 mmu).[6,7] The ^{1}H NMR spectral data of **3** resembled those for **2**, except at C17 (δ_H 3.95 ppm).[5,10] The reduced compound **2** was treated with $NaBH_4$ to give compound **3**. All of the signals were assigned by a detailed comparison of NMR spectral data with those of **2** and by ^{1}H-^{1}H COSY spectra. The chemical shift at C17 (δ_C 75.2 ppm) and the molecular formula of **3** indicated the presence of a hydroxyl group at C17. Furthermore, **3** was treated with acetic anhydride and pyridine, and the corresponding monoacetate (**4**) was obtained. The stereochemistry at C17 was suggested by the coupling constants of **4**. H17 was assigned to be axial based on the relatively large coupling constants ($J_{17,18}$= 8.8 Hz), as shown in Figure 3.[11] These data indicated the relative stereochemistry of **3**.

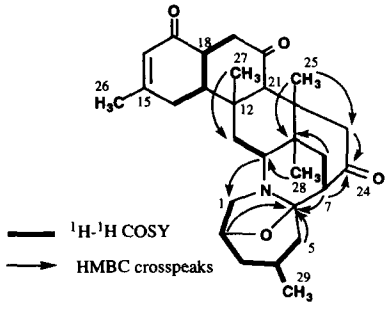

Figure 2. Planar Structure and HMBC Crosspeaks of **2**.

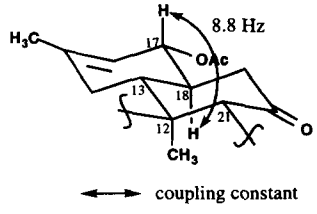

Figure 3. Relative Stereochemistry at C12-C22 Moiety of **4**.

Treatment of **3** with (-)- and (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl) gave the (*S*)- and (*R*)-MTPA esters (**5** and **6**), respectively.[12] The ^{1}H NMR chemical shifts of **5** and **6** were assigned based on a detailed analysis of ^{1}H-^{1}H COSY spectral data. The differences in the chemical shift ($\Delta\delta$; δ_S-δ_R) in the ^{1}H NMR spectra are shown in Figure 4. Positive $\Delta\delta$ values were observed for H10, H19a, H19b, H21, H23a, H23b, H25, H27, and H28, whereas negative values were observed for H16, H17, and H26, indicating that the absolute configuration of **3** at C17 was *S*. These data and the relative stereochemistry of **2** suggested that the absolute configuration of reduced compound **3** at C2, C4, C6, C7, C9, C10, C12, C13, C17, C18, C21, and C22 was *R*, *S*, *R*, *S*, *S*, *R*, *S*, *R*, *S*, *S*, *S*, and *S*. Therefore, the absolute stereochemistry of norzoanthamine was deduced to be 2*R*, 4*S*, 6*S*, 9*S*, 10*R*, 12*S*, 13*R*, 18*S*, 21*S*, and 22*S*.

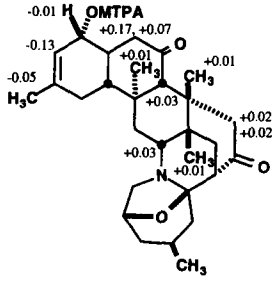

Figure 4. $\Delta\delta$ Values [$\Delta\delta$ (in ppm)= δ_S-δ_R] Obtained for (*S*)- and (*R*)-MTPA Esters of **3**.

Finally, the absolute stereochemistry of norzoanthamine was completely determined, as shown in **1**. Furthermore, zoanthamine, oxyzoanthamine, norzoanthamininone, cyclozoanthamine, and epinorzoanthamine norzoanthamine-related alkaloids isolated from the colonial zoanthid *Zoanthus* sp. have the same absolute stereochemistry as that of norzoanthamine.

Although zoanthamines have been regarded as terpenoids based on their molecular formulas, the biogenetic pathway of zoanthamines is unclear. We propose here a polyketide biogenetic pathway for zoanthamines, as shown in Figure 5.

The mechanism of action and the *in vivo* behaviors of the samples are currently under investigation. Further studies on the detailed chemistry of norzoanthamine, including the biogenetic pathway and structure-activity relationships, are underway in our laboratory.

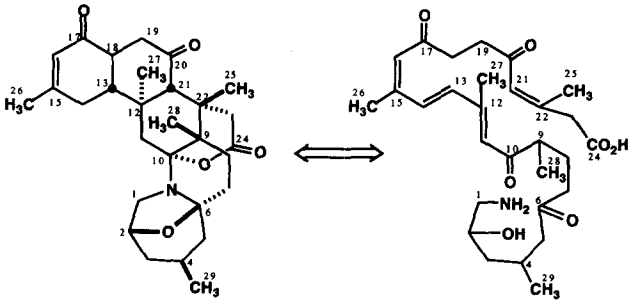

Figure 5. Proposed Biogenetic Pathway of Norzoanthamine.

Acknowledgments

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5. NMR spectra were recorded on a JNM-GSX 400 spectrometer.
6. Mass spectra were recorded on a M-80B mass spectrometer.
7. **2**: IR ($CHCl_3$) 2950, 1700, 1660, 1600 cm^{-1}, $[\alpha]_D$= -33° (*c* 0.71, $CHCl_3$); **3**: IR ($CHCl_3$) 3450, 2950, 1700 cm^{-1}, $[\alpha]_D$= -18° (*c* 0.17, $CHCl_3$).
8. ^1H NMR Data of **2** in $CDCl_3$: δ 2.78 (1H, dd, *J*= 10.6 and 7.3, H-1a), 3.23 (1H, dd, *J*= 10.7 and 1.5, H-1b), 4.50(1H, m, H-2), 1.30 (1H, ddd, *J*= 13.4, 11.0, and 2.5, H-3a), 1.46 (1H, ddd, *J*= 13.4, 5.1, and 3.6, H-3b), 1.98 (1H, m, H-4), 1.07 (1H, dd, *J*= 13.2 and 12.5, H-5a), 1.40 (1H, dd, *J*= 12.5 and 5.8, H-5b), 2.70 (1H, dd, *J*= 3.3 and 2.9, H-7), 1.84 (1H, dd, *J*= 14.1 and 3.3, H-8a), 1.90 (1H, dd, *J*= 14.1 and 2.9, H-8b), 2.58 (1H, dd, *J*= 2.2 and 4.5, H-10), 1.70 (1H, dd, *J*= 14.2 and 4.5, H-11a), 1.79 (1H, dd, *J*= 14.2 and 2.2, H-11b), 2.09 (1H, ddd, *J*= 14.6, 8.5, and 6.2, H-13), 2.32 (1H, dd, *J*= 13.5 and 8.5, H-14a), 2.33 (1H, dd, *J*= 13.5 and 6.2, H-14b), 5.89 (1H, s, H-16), 2.58 (1H, ddd, *J*= 14.6, 11.8 and 6.9, H-18), 2.34 (1H, dd, *J*= 13.1 and 11.8, H-19a), 2.69 (1H, dd, *J*= 13.1 and 6.9, H-19b), 2.53 (1H, s, H-21), 3.15 (1H, d, *J*= 14.2, H-23a), 3.46 (1H, d, *J*= 14.2, H-23b), 0.99 (3H, s, H-25), 2.00 (3H, s, H-26), 1.39 (3H, s, H-27), 0.90 (3H, s, H-28), 0.84 (3H, d, *J*= 6.6, H-29); ^{13}C NMR Data of **2** in $CDCl_3$: δ 60.1 (C-1), 72.2 (C-2), 37.9 (C-3), 23.3 (C-4), 44.6 (C-5), 92.4 (C-6), 54.7 (C-7), 33.6 (C-8), 36.3 (C-9), 71.1 (C-10), 36.4 (C-11), 41.2 (C-12), 53.5 (C-13), 31.3 (C-14), 160.0 (C-15), 125.5 (C-16), 198.7 (C-17), 45.5 (C-18), 43.0 (C-19), 209.0 (C-20), 62.2 (C-21), 41.1 (C-22), 47.1 (C-23), 211.5 (C-24), 22.7 (C-25), 24.2 (C-26), 17.7 C-27), 24.0 (C-28), 21.7 (C-29).
9. Quite recently, we performed an X-ray crystallographic analysis of **2** itself. Detailed data will be reported elsewhere.
10. ^1H NMR Data of **3** in $CDCl_3$: δ 2.78 (1H, dd, *J*= 10.7 and 7.3, H-1a), 3.24 (1H, dd, *J*= 10.7 and 0.7, H-1b), 4.50 (1H, dddd, *J*= 10.7 and 7.3, 2.1, 1.9, and 0.7, H-2), 1.33 (1H, ddd, *J*= 13.3, 9.2, and 2.1, H-3a), 1.45 (1H, ddd, *J*= 13.3, 4.8, and 1.9, H-3b), 1.98 (1H, qdddd, *J*= 6.6, 12.3, 9.2, 5.5, and 4.8, H-4), 1.09 (1H, dd, *J*= 13.3 and 12.3, H-5a), 1.44 (1H, dd, *J*= 13.3 and 5.5, H-5b), 2.71 (1H, dd, *J*= 3.3 and 2.7, H-7), 1.85 (1H, dd, *J*= 14.1 and 2.7, H-8a), 1.95 (1H, dd, *J*= 14.1 and 3.3, H-8b), 2.56 (1H, m, H-10), 1.75 (1H, m, H-11a), 1.78 (1H, m, H-11b), 1.78 (1H, m, H-13), 1.72 (1H, m, H-14a), 1.95 (1H, m, H-14b), 5.38 (1H, br. s, H-16), 3.95 (1H, br.s, H-17), 1.75 (1H, m, H-18), 2.09 (1H, dd, *J*= 14.0 and 4.6, H-19a), 2.83 (1H, dd, *J*= 14.0 and 10.3, H-19b), 2.60 (1H, s, H-21), 3.18 (1H, d, *J*= 13.9, H-23a), 3.53 (1H, d, *J*= 13.9, H-23b), 1.01 (3H, s, H-25), 1.73 (3H, s, H-26), 1.33 (3H, s, H-27), 0.91 (3H, s, H-28), 0.85 (3H, d, *J*= 6.6, H-29); ^{13}C NMR Data of **3** in $CDCl_3$: δ 60.1 (C-1), 71.1 (C-2), 37.8(C-3), 23.2 (C-4), 44.5 (C-5), 92.5 (C-6), 54.7 (C-7), 33.6 (C-8), 36.1 (C-9), 71.1 (C-10), 36.1 (C-11), 41.2 (C-12), 50.8 (C-13), 29.4 (C-14), 133.0 (C-15), 124.8 (C-16), 75.2 (C-17), 42.5 (C-18), 47.5 (C-19), 210.0 (C-20), 62.0 (C-21), 41.1 (C-22), 47.1 (C-23), 212.0 (C-24), 22.8 (C-25), 23.3 (C-26), 17.3 C-27), 24.1 (C-28), 21.8 (C-29).
11. The monoacetate of **3** was obtained by treating it with Ac_2O/pyridine at r.t. for 12 hr. The resulting monoacetate was purified by preparative TLC on SiO_2 with 1.5 % $CH_3OH/CHCl_3$. The ^1H NMR spectrum was measured in $CDCl_3$. Chemical shifts of H17 and H2' (-OOC$\underline{CH_3}$) in the ^1H NMR spectral data of monoacetate of **3** are as follows: H17 (δ_H 5.22 ppm, $J_{H17,18}$= 8.8 Hz), H2' (δ_H 2.04 ppm).
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