

Confirmation of the Structure of Oxystemokerrin by Single Crystal X-Ray Structural Analysis and a Proposed Biosynthesis

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Abstract

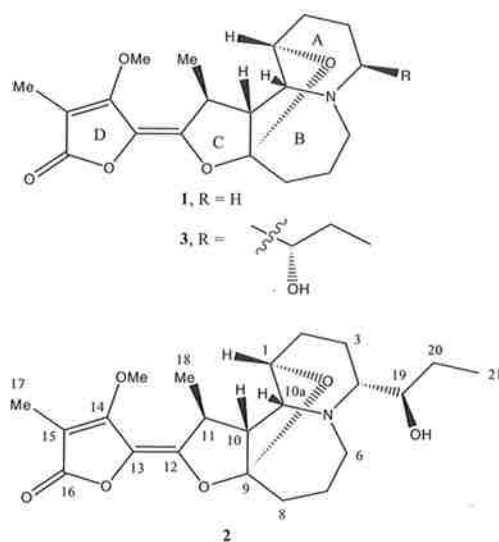
The crystal structure of the pentacyclic *Stemona* alkaloid oxystemokerrin (**2**), isolated from the roots of *Stemona kerrii*, is reported, confirming the structure proposed for this compound in a contemporaneous spectroscopic study. This compound is a diastereomer of stemocurtisinol (**3**), whose structure we have recently reported from *S. curtisii*, also confirmed by a single-crystal X-ray study. These alkaloids have opposite configurations at C-4 and C-19. A possible biosynthetic pathway for the biosynthesis of **2** and **3** is proposed.

Keywords: X-ray structure, oxystemokerrin, *Stemona*, alkaloid

Introduction

The *Stemona* group of alkaloids includes more than forty different natural products that have been structurally classified into five different groups.¹ The pyrrolo[1,2-*a*]azepine (5,7-bicyclic A,B-ring system) nucleus is common to all compounds in these groups. In 2003 we reported the structure of stemocurtisine (**1**), the first example of a *Stemona* alkaloid with a pyrido[1,2-*a*]azepine A,B-ring system (that is, a 6,7-bicyclic A,B-ring system), isolated from the roots of *S. curtisii* Hook. f.² Later in that year Hofer and Greger reported the isolation of five *Stemona* alkaloids with the pyrido[1,2-*a*]azepine A,B-ring system, including stemocurtisine (**1**), which they named pyridostemin, and oxystemokerrin (**2**) from an unidentified *Stemona* species (HG 915) and *S. kerrii*, respectively.³ The structure of the latter compound was deduced from spectral interpretation and by analogy to the structurally related alkaloid, stemokerrin, for which the structure was secured by an X-ray crystallographic study. In 2004 we reported the single-crystal structure of stemocurtisinol (**3**), a diastereomer of **2**, having the opposite configurations at C-4 and C-19 and correspondingly different spectral characteristics.⁴

Our sample of oxystemokerrin was isolated from the root extracts of *S. kerrii* that were collected at Tambol Mae Hea, Amphur Maung, Chiang Mai, Thailand, in August 2003. The plant material was identified by Mr. James F. Maxwell from the Department of Biology, Chiang Mai University, where a voucher specimen has been lodged (number 17584). After purification by column chromatography, compound **2** was obtained as colourless prismatic crystals (mp 134-136 °C) by careful and slow evaporation of a solution of **2** in ethanol.



The single crystal structure of oxystemokerrin (**2**) is shown in Figure 1 (a single molecule, devoid of crystallographic symmetry comprising the asymmetric unit), clearly establishing that the structure and relative stereochemistry proposed for this compound by Hoger and Greger is correct.⁵ The conformational descriptors of the fused ring system of **2** differ only slightly from those of **3** (Table 1); the hydroxyl hydrogen interacts intramolecularly with N(5) (Figure 1).

While biosynthetic studies on *Stemona* alkaloids have not been reported, a proposed biosynthetic pathway leading to the pyrrolo[1,2-*a*]azepine *Stemona* alkaloids has been made by Seger *et al.*⁶ The terpenoid origin of the C- and D-ring carbons has been postulated by Seger, while the A-ring of these alkaloids has been postulated to arise from spermidine. A ring expansion of the pyrrolidine ring (A-ring) to a piperidine ring has been proposed

to account for the biosynthesis of the pyrido[1,2-*a*]zepine *Stemona* alkaloids.³ Another possibility for formation of the A-ring of oxystemokerrin (**2**) and stemocurtisinol (**3**) is shown in Scheme 1. Our proposed biosynthesis involves an alternative biosynthesis of the A-ring of **2** and **3** based on the known biosynthesis of the hemlock alkaloid (+)-conhydrine **4** from the acetate-derived polyketide derivative **5**.^{7,8} Condensation of **5** with 1,4-diaminopropane, a biosynthetic product from the homospermidine synthase (HSS) production of homospermidine,⁹ could provide the piperidine A-ring precursor intermediate **6** (Scheme 1). A stereoselective reduction of the cyclic iminium ion intermediate **6** and a stereoselective oxidation at C-1 of the propyl side-chain of **6** may lead to intermediate iminium ion **7**. Coupling of **7** to a geranyl unit, as proposed by Seger *et al.*,⁶ could then provide alkaloids **2** or **3** (Scheme 1). Whatever the biosynthetic pathway, clearly at least three *Stemona* species of plants have evolved to produce enzymes that give opposite stereochemical outcomes in their biosynthetic reactions to produce stereoselectively either oxystemokerrin (**2**) or stemocurtisinol (**3**).

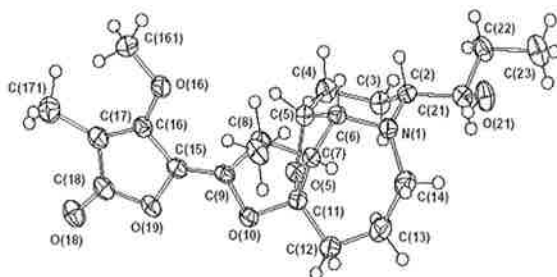


Figure 1. Molecular projection of **2**, showing 50% probability amplitude displacement ellipsoids for the non-hydrogen atoms, hydrogen atoms having arbitrary radii of 0.1 Å.

Experimental

Plant material

The root of *S. kerrii* were collected at Tambol Mae Hea, Amphur Maung, Chiang Mai, Thailand, in August 2003. The plant material was identified by Mr. James F. Maxwell from the Department of Biology, Chiang Mai University, where a voucher specimen is lodged (number 17584).

Extraction and isolation

The dry ground root of *S. kerrii*. (2.5 kg) was extracted with 95% ethanol (3 x 2000 mL) over 3 days at room temperature. The ethanolic solution was evaporated to give a dark residue (139 g). A

portion of the extract (50 g) was partitioned between water and dichloromethane (DCM). The DCM extract was extracted with 5% HCl solution, and the aqueous solution was made basic with aqueous ammonia and extracted with DCM to afford 1.4 g of crude alkaloid material. This material was chromatographed on silica gel (120 mL) using gradient elution from 100% DCM to 25% MeOH/DCM containing 1% conc. aqueous ammonia as eluent. A total of 1.1 L of eluent was collected in test tubes of 20 mL. On the basis of TLC analysis tubes 14-17 were pooled and evaporated and the resulting mixture (661 mg) was re-chromatographed as described above. Tubes 25-26 were pooled together to give a sample of oxystemokerrin (**2**) (90 mg). The ¹H and ¹³C NMR and MS data of **2** are identical to that reported in the literature.³ Compound **2** was obtained as colourless prismatic crystals (mp. 134-136 °C) by careful and slow evaporation of a solution of **2** in ethanol.

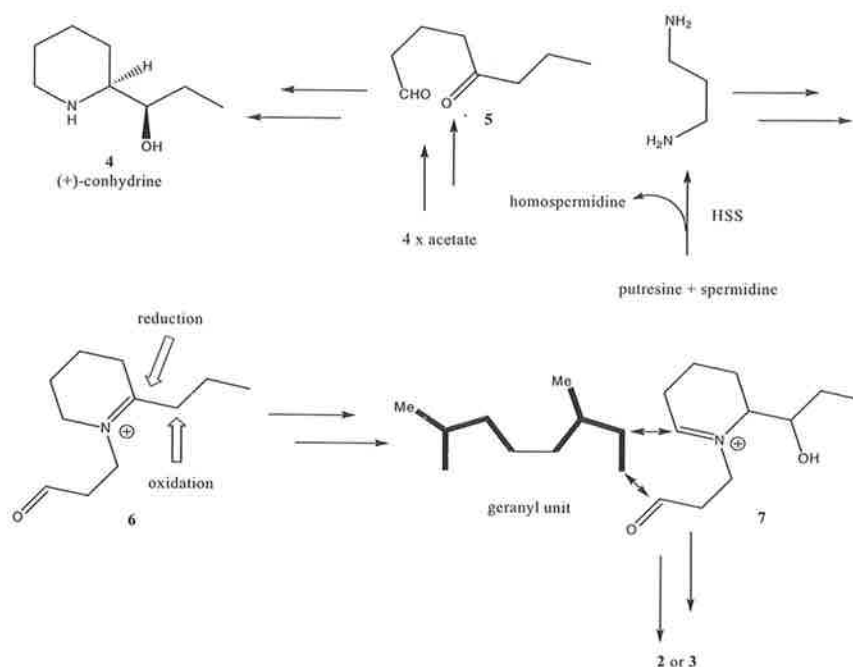
Structure determination

A full sphere of 'low'-temperature CCD diffractometer data was measured (Bruker AXS instrument; ω -scans, $2\theta_{\max} = 58^\circ$; monochromatic Mo K α radiation, $\lambda = 0.71073 \text{ \AA}$; $T \text{ ca. } 153 \text{ K}$) yielding 10321 reflections, these merging to 2825 unique ($R_{\text{int}} 0.041$) after 'empirical'/multiscan absorption correction ('Friedel' data also merged, there being no non-trivial anomalous scatterer); 2184 with $F > 4\sigma(F)$ were considered 'observed' and used in the full-matrix least squares refinement, refining anisotropic displacement parameter forms for C, N, O, (x, y, z, U_{iso})H being included constrained at estimates. Conventional residuals on $|F|$ are 0.060, 0.063, reflection weights being $(\sigma^2(F) + 0.0004 F^2)^{-1}$. Neutral atom scattering factors were employed, computation using the Xtal 3.7 program system.¹⁰ The setting of the chirality follows the known chemistry.

Crystal data: C₂₂H₃₁NO₆, $M = 405.5$.

Monoclinic, space group $P2_1$ (C_2^2 , No. 4), $a = 9.148(3)$, $b = 10.530(3)$, $c = 11.152(4) \text{ \AA}$, $\beta = 103.711(5)^\circ$, $V = 1044 \text{ \AA}^3$. D_c ($Z = 2$) = 1.290 cm^{-3} .

$\mu_{\text{Mo}} = 0.093 \text{ mm}^{-1}$; specimen: 0.24 x 0.18 x 0.08 (diffracting poorly). $|\Delta\rho_{\max}| = 0.70(4)$.

Scheme 1. Proposed biosynthesis of alkaloids **2** and **3**.**Table 1.** Fused ring torsion angles (degrees) of **2** (Atoms are denoted by number only, *N*, *O*, italicized)

Atoms	Angle	Atoms	Angle
12-9-9-10	31.8(5), 28.9(2)	10a-5-6-7	50.6(7), 55.0(3)
9-9-10-11	-18.8(5), -20.9(2)	5-6-7-8	-71.1(7), -75.5(3)
9-10-11-12	0.7(5), 6.4(2)	6-7-8-9	51.1(6), 52.1(3)
10-11-12-9	19.0(5), 11.2(2)	7-8-9-10	-67.9(6), -64.4(3)
11-12-9-9	-32.5(5), -25.6(2)	8-9-10-10a	99.4(5), 96.3(2)
		9-10-10a-5	-81.7(4), -83.5(2)
10a-1-2-3	40.2(6), 37.2(8)	10-10a-5-6	22.6(6), 21.0(3)
1-2-3-4	-47.3(6), -44.9(3)		
2-3-4-5	60.4(5), 56.1(2)	9-1-1-10a	28.7(4), 24.1(2)
3-4-5-10a	-62.9(4), -58.4(2)	1-1-10a-10	-42.1(3), -38.6(2)
4-5-10a-1	51.9(5), 49.0(2)	1-10a-10-9	39.4(4), 38.4(2)
5-10a-1-2	-42.6(6), -38.9(2)	10a-10-0-1	-23.7(4), -25.5(2)
		10-9-1-1	-3.2(4), 0.6(2)

Acknowledgements

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