Synthesis of 10b-*R*-Hydroxy-pancratistatin *via* Narciclasine

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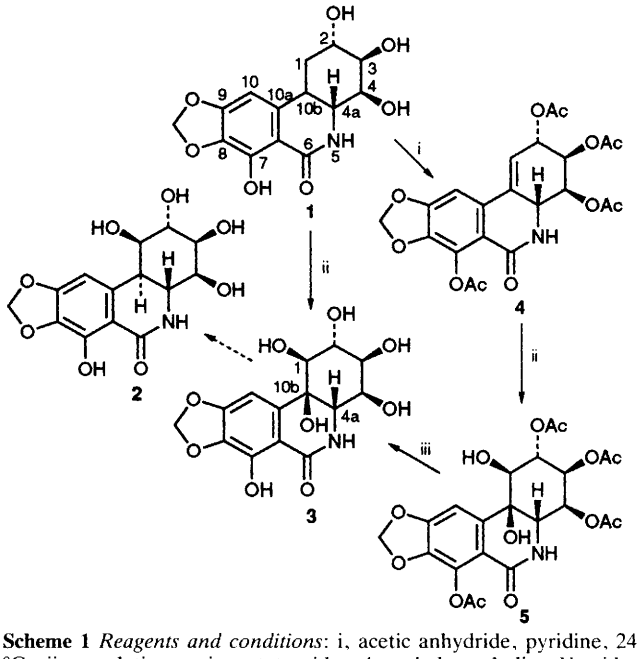
A diastereoselective synthesis of 10b-*R*-hydroxy-pancratistatin **3** from narciclasine **1** has been achieved.

Traditional medicinal applications recorded over more than a millennium[1b] have been the impetus for many studies of amaryllidaceous plants,[2a–f] resulting in the identification of a large family of structurally related phenanthridone constituents such as narciclasine[2a] **1**, narciprimine,[2b] trans-dihydro-narciclasine,[2c] 7-deoxy-narciclasine,[2d] 7-deoxy-*trans*-dihydro-narciclasine[2e] and pancratistatin,[1a,2d] **2**. The latter isocarbostyril was isolated[2d,3] from the bulbs of *Hymenocallis* (formerly *Pancratium*) *littoralis* (Jacq.) native to Hawaii in relatively low (0.0019%) yields, and is undergoing preclinical development as an anticancer[4a] and antiviral drug.[4b,c] Consequently, pancratistatin has become a very important, and surprisingly difficult, synthetic target.[5] The six contiguous asymmetric centres in the *C*-ring of the phenanthridone skeleton adds to the challenge. To date, only one total synthesis of pancratistatin has been reported.[6] Although a considerable achievement, this synthesis only provided racemic pancratistatin in 0.13% overall yield *via* 26 steps. Therefore, isolation from plants still remains the only practical route to the multigram quantities of this valuable compound necessary for eventual clinical trials.

An attractive solution to the preclinical supply problems encountered with pancratistatin would be a high yield partial synthesis from narciclasine **1** already available in multigram quantities from the bulbs of various Amaryllidaceae species. We now report a synthetic approach to pancratistatin **2** starting from narciclasine **1** that has resulted in the synthesis of 10b-(*R*)-hydroxy-pancratistatin **3**. Narciclasine **1** was isolated in multigram quantities by scale-up isolation from a *Narcissus sp.*[4] and allowed to react under the special Sharpless[7] osmium tetroxide conditions to give exclusively the diol derivative **3** (72% yield), Scheme 1. When hydroquinine 4-chlorobenzoate (HQ) was replaced with its diastereoisomer hydroquinidine 4-chlorobenzoate (HQD) diol **3** (dp 269 °C) remained as the only product detected. The stereochemical result corresponds to Kishi's[7] predictions that approach of osmium tetroxide to the face of the olefinic bond is opposite to that of a pre-existing hydroxy group in an allylic alcohol. The presence of either chiral ligand, (HQ or HQD), failed to induce the formation of 10b-(*S*)-hydroxy-isopancratistatin. Acetylation of narciclasine **1** afforded tetraacetate **4** (80% yield) which when osmylated (Scheme 1) gave exclusively diol **5** (76% yield). Deacetylation using a methanolic ammonia solution led to alcohol **3**.

Detailed analysis of the NMR 2D COSY, ^{1}H–^{1}H correlation spectrum completed assignment of the hydrogen atom resonances for the 1D NMR spectrum. Both deuterium exchange and highfield (400 MHz) homonuclear decoupling experiments permitted measurement of the coupling constant ($J_{1,2}$ = 9 Hz) for alcohol **3**. Interpretation of further NMR spectral results suggested introduction of a β-*cis*-diol into the phenanthridone at the 1 and 10b positions. That assumption was confirmed by an X-ray crystal structure determination† of the acetate derivative **5**. A suitable crystal (0.08 × 0.14 × 0.40 mm) was grown from methanol. The structure was solved with the direct methods program SIR88.[8b] All non-hydrogen atoms, with the exception of the aromatic acetate group, were located on the first run of SIR88, using the default settings. Subsequent difference Fourier maps revealed considerable disorder in the region of the phenol acetate at C-7. The disorder was finally resolved by choosing two alternate rotational isomers for the acetate unit (Fig. 1), each of which was assigned 0.50 occupancy. All non-hydrogen atoms were refined[8a] anisotropically, with the exception of the aromatic acetates which were refined isotropically by full-matrix least squares methods. All hydrogen atom coordinates were calculated at optimum positions and then forced to ride the atoms to which they were attached, but were not refined. The refinement of 322 parameters converged at R = 0.0552 for 1690 observed reflections. The largest difference peak and hole in the final difference Fourier were 0.436 and −0.297 e Å^{-3} respectively.

The X-ray molecular structure of 10b-(*R*)-hydroxy-pancratistatin tetraacetate **5** is shown in Fig. 1. The two hydroxy



Scheme 1 *Reagents and conditions*: i, acetic anhydride, pyridine, 24 °C; ii, catalytic osmium tetroxide, 4-methylmorpholine-*N*-oxide, hydroquinine 4-chlorobenzoate, 9:1 *v/v* dimethylformamide–H_2O, 0–24 °C; iii, methylene chloride, 2 mol dm^{-3} ammonia in methanol, 24 °C

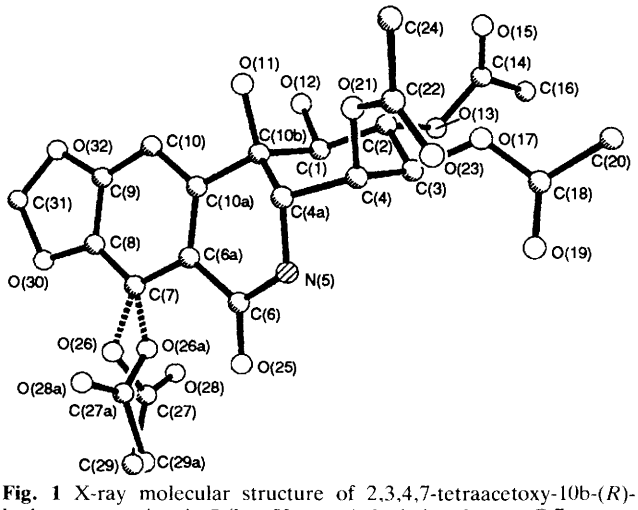


Fig. 1 X-ray molecular structure of 2,3,4,7-tetraacetoxy-10b-(*R*)-hydroxypancratistatin **5** (less H atoms) depicting the two C-7 acetate rotamers

groups in the 1- and 10b-positions are on the β-face. As noted above, the phenol acetate unit attached to C-7 seemed to exhibit considerable disorder. Rather than being coplanar with the aromatic ring as expected it was found to deviate from coplanarity with the aromatic *A*-ring by +18.9° and −24.7°. These two alternate acetate sites exhibit the characteristics of conformational isomers (rotamers) as a result of substantial steric hindrance between the carbonyl oxygen of the acetate and the carbonyl of the lactam on the adjacent *B*-ring. As a result, two stable rotamers can be envisioned, Fig. 1.

After the large number of attempts to hydroxylate or otherwise selectively oxidize the 1,10b-olefin system, we were pleased to observe the totally diastereoselective osmylation of olefins **1** and **4** to afford alcohols **3** and **5** respectively. Houk has suggested[11] staggered models for the transition state structures, based on *ab initio* calculations, for the reaction of allylic alcohols[10,7] with osmium tetroxide. The allylic groups play decisive roles in obtaining the high degree of stereoselectivity.

Significantly, the cancer cell growth inhibitory activity of 10b-(*R*)-hydroxy-pancratistatin **3** against the P388 lymphocytic murine leukaemia and a selection of human cancer cell lines was found to be about 1000 times less potent than pancratistatin **2**. Apparently, the added steric features of the 10b-β-hydroxy group causes a pronounced reduction in cancer cell growth inhibition.

Presently, we are investigating a number of methods[12] for cleavage of the 10b-benzyl alcohol by hydrogenolysis that will complete this potentially practical synthetic route to pancratistatin. Furthermore, employing narciclasine as a relay would complete a formal total synthesis of pancratistatin. However, the superficially simple final step involving benzyl–oxygen bond cleavage has, to date, proved refractory and will require further extensive study.

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Footnote

† *Crystal data* for compound **5**: $C_{22}H_{23}NO_{13}$, M = 509.41, orthorhombic, a = 9.096(1), b = 9.799(2), c = 25.570(5) Å, V = 2279.10 Å^3, space group $P2_12_12_1$, D_m = 1.467 g cm^{-3}, D_c = 1.485 g cm^{-3}, Z = 4, $F(000)$ = 1064, graphite-monochromated Cu-Kα radiation, λ = 1.54184 Å, μ = 1.074 mm^{-1}. The intensities of 1891 independent reflections with 2θ ≤ 120° were measured on an Enraf-Nonius CAD4 diffractometer at 26 °C using the ω/2θ scan technique, of which 1690 reflections with $I > 2\sigma(I_o)$ were used in refinement.[8a] Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

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