# Two Unique Pentacyclic Steroids with Cis C/D Ring Junction from *Xestospongia bergquistia* Fromont, Powerful Inhibitors of Histamine Release

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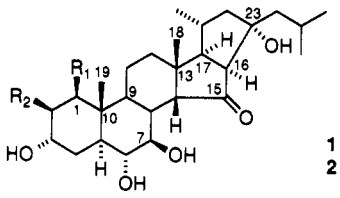
*Summary:* Xestobergsterol A (1) (23*S*-16$\beta$,23-cyclo-3$\alpha$,6$\alpha$,7$\beta$,23-tetrahydroxy-5$\alpha$,14$\beta$-cholestan-15-one) and B (2) (23*S*-16$\beta$,23-cyclo-1$\beta$,2$\beta$,3$\alpha$,6$\alpha$,7$\beta$,23-hexahydroxy-5$\alpha$,14$\beta$-cholestan-15-one), potent inhibitors of histamine release from rat mast cells induced by anti-IgE, are the first report of steroids with both the $C_{16}/C_{23}$ bond and cis C/D ring junction.

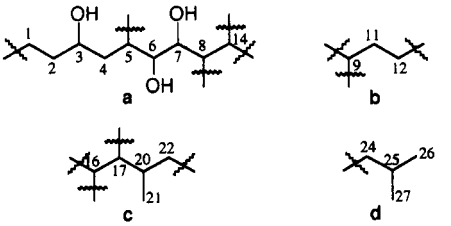
Marine organisms have been the source of many steroids, and a number of groups have been involved chemically and pharmacologically.[1] In our search for pharmacologically active substances from marine organisms, we found that the crude extract of the Okinawan marine sponge *Xestospongia bergquistia* Fromont[2] strongly inhibited histamine release from rat mast cells induced by anti-IgE.[3] Bioassay-guided purification resulted in the isolation of two unique pentacyclic steroids, named xestobergsterol A (1) and B (2). We now report the isolation and structure elucidation of the two compounds.



1 $R_1,R_2$=H
2 $R_1,R_2$=OH

The methanol/toluene extract of the sponge was divided into the ethyl acetate-, *n*-butanol-, and water-soluble portions. The activity was found in the first two portions, which were combined and subjected to silica gel medium-pressure column chromatography, followed by Sephadex LH-20 column chromatography. The active fractions were further purified by reversed-phase HPLC to give 1 (530 mg, 0.059% wet weight) and 2 (120 mg, 0.013%).

Xestobergsterol A (1) was obtained as an amorphous white powder.[4] The molecular formula, $C_{27}H_{44}O_5$, which was determined by mass measurement [FABMS (M + Na)$^+$ 471, HREIMS, and LREIMS], implied six degrees of unsaturation. The number of the carbon atoms and the positive Liebermann-Burchard reaction suggested a steroid for 1. The UV spectrum showed only end absorptions. An IR band at 1725 cm$^{-1}$ and a $^{13}$C NMR signal at $\delta$ 217.3 indicated the presence of a carbonyl group in the five-membered ring. Since resonances in the $^{13}$C NMR spectrum indicated no double bonds, the carbon skeleton consists of five rings. The $^1$H NMR spectrum contained two methyl singlets ($\delta$ 0.89 and 1.18) and three methyl doublets ($\delta$ 1.04, 1.06, and 1.12). Interpretation of the COSY experiment of 1 led to four partial structures, **a**–**d**.



The HMBC experiment revealed that the $H_{19}$ methyl protons were coupled to $C_1$, $C_5$, $C_9$ and $C_{10}$, the $H_8$ methine proton to $C_9$, the $H_{14}$ methine proton to $C_8$, and the $H_9$ methine proton to $C_{14}$. This suggested a linkage between partial structures **a** and **b**. In the HMBC spectrum there were cross-peaks appearing from $H_{18}$ to $C_{12}$, $C_{13}$, $C_{14}$, and $C_{17}$, $H_{14}$ to $C_{12}$, $H_{17}$ to $C_{13}$, and $H_7$ to $C_{14}$. These connectivities established the connection between the partial structures **b** and **c**. Furthermore, the HMBC spectrum exhibited couplings between $H_{16}$ and $C_{23}$, $H_{22}$ and $C_{16}$, $H_{22}$ and $C_{23}$, $H_{22}$ and $C_{24}$ $H_{24}$ and $C_{16}$, $H_{24}$ and $C_{22}$, and $H_{24}$ and $C_{23}$. These data established the connectivity between the partial structures **c** and **d**, which accounted for the presence of another C–C bond between $C_{16}$ and $C_{23}$. A long-range correlation between the carbonyl carbon and $H_{14}$, $H_{16}$, and $H_{17}$ established the site of the carbonyl at $C_{15}$.

The relative stereochemistry of 1 was determined by difference NOE experiments and coupling constants. NOE experiments clearly defined the usual chair conformation and trans junctional of rings A and B. The hydroxyl group

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(2) The sponge was identified as *Xestospongia bergquistia* Fromont by Professor P. R. Bergquist, Department Zoology, School of Biological Sciences, The University of Auckland, Auckland, New Zealand.

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(4) $^1$H NMR (400 MHz, pyridine-$d_5$) $\delta$ 1.76 (H1), 1.50 (H1), 2.72 (H2), 1.65 (H2), 4.35 (H3), 1.88 (H4), 1.68 (H4), 2.36 (H5), 3.57 (dd, $J$ = 10.0, 10.0 Hz, H6), 5.05 (dd, $J$ = 10.0, 10.0 Hz, H7), 1.97 (H8), 1.57 (H9), 1.54 (H11), 1.24 (H11), 1.41 (H12), 1.06 (H12), 3.42 (H14), 2.70 (d, $J$ = 9.5 Hz, H16), 1.72 (H17), 1.18 (s, H18), 0.89 (s, H19), 2.63 (H20), 1.12 (d, $J$ = 6.6 Hz, H21), 2.12 (dd, $J$ = 12.1, 5.9 Hz, H22), 1.33 (dd, $J$ = 12.1, 12.1 Hz, H22), 1.98 (H24), 1.56 (H24), 2.19 (H25), 1.06 (d, $J$ = 5.1 Hz, H26), 1.04 (d, $J$ = 5.1 Hz, H27); $^{13}$C NMR (100 MHz, pyridine-$d_5$) $\delta$ 33.1 (t, C1), 31.3 (t, C2), 65.3 (d, C3), 29.4 (t, C4), 42.4 (d, C5), 75.7 (d, C6), 74.9 (d, C7), 39.1 (d, C8), 46.5 (d, C9), 36.9 (s, C10), 21.5 (t, C11), 38.6 (t, C12), 38.4 (s, C13), 51.7 (d, C14), 217.3 (s, C15), 62.8 (d, C16), 58.0 (d, C17), 19.9 (q, C18), 12.8 (q, C19), 34.9 (d, C20), 20.9 (q, C21), 52.3 (t, C22), 82.2 (s, C23), 52.0 (t, C24), 25.0 (d, C25), 24.9 (q, C26), 25.3 (q, C27).

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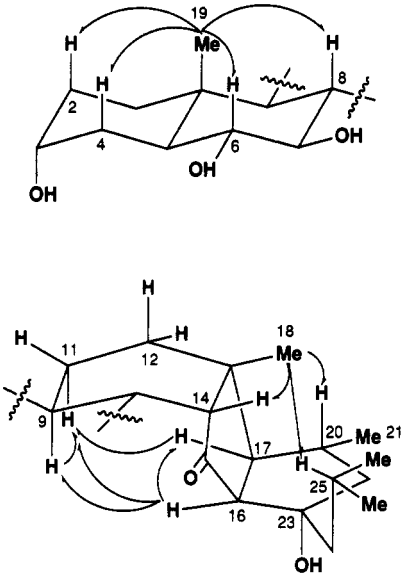
in $C_3$ was confirmed to be $\alpha$-oriented from the broad singlet-like signal at $\delta$ 4.35 and the upfield shift of the $C_3$ signal ($\delta$ 65.3). The coupling constants of $H_6$ (dd, $J$ = 10.0, 10.0 Hz) and $H_7$ (dd, $J$ = 10.0, 10.0 Hz) determined the $\alpha$- and $\beta$-oriented hydroxyl groups, respectively. The cis C/D ring junction was determined as follows: $H_{14}$ appeared as a broad singlet-like signal at $\delta$ 3.42 and the NOE was observed between $H_{18}$ and $H_{14}$ but not between $H_{16}$ and $H_{14}$. The NOE between $H_{18}$ and $H_{20}$ indicated the $C_{20}$ $R$ configuration. The two $\alpha$-oriented hydrogens at $C_{16}$ and $C_{17}$ were confirmed by the NOE between $H_{16}$ and $H_{17}$ and $H_{16}$ and $H_9$. The NOE was detected between $H_{18}$ and $H_{25}$, but not between $H_{21}$ and $H_{25}$, confirming the $C_{23}$ $S$ configuration. Furthermore, the NOE was observed between $H_{17}$ and $H_{11}$, $H_{16}$ and $H_{11}$, and $H_9$ and $H_{11}$, but not between $H_9$ and $H_{12}$, indicating a boat conformation of the ring C.




NOE correlations for 1

Xestobergsterol B (2) was obtained as an amorphous white powder.[5] The molecular formula of 2 was determined to be $C_{27}H_{44}O_7$ (FABMS $(M + Na)^+$ 503, HREIMS, and LREIMS), differing from the molecular formula of 1 in addition of $O_2$. Comparison of physicochemical data of 2 with those of 1 revealed that the only difference was 2 having hydroxyl groups at the $C_1$ and $C_2$ positions. These aspects were confirmed by six carbon signals connected to oxygen atoms in the $\delta$ 65–82 region in the $^{13}C$ NMR spectrum. The connectivity of the COSY and HMBC experiments supported the assumed structure of 2.

The NOE between $H_9$ and $H_1$ established the $\beta$-hydroxyl group in the $C_1$ position. The $\beta$-hydroxyl group configuration in $C_2$ was determined by the broad singlet-like signal at $\delta$ 4.46. The other configurations of asymmetric carbons were determined by NOE experiments and coupling constants and found to be the same as in 1.

Biogenetically, 1 and 2 are considered most likely to be produced by an intramolecular aldol-type reaction in the organism. The possibility that 1 and 2 are artifacts arising during the isolation process was excluded, because silica gel TLC of the original methanol/toluene extract showed the presence of 1 and 2 as major spots and because no base or no acid was employed in the present experiment. Very recently, R. J. Andersen et al. reported contignasterol from the sponge *Petrosia contignata* as the first steroid with cis C/D ring junction from marine organisms.[6] To the best of our knowledge, this is the first isolation of steroids posessing five carbocyclic rings and cis C/D ring junction.

In the present study, 1 and 2 strongly inhibited histamine release from rat peritoneal mast cells induced by anti-IgE in the dose-dependent manner.[7] The $IC_{50}$ values of 1 and 2 were 0.05 and 0.10 $\mu$M, respectively. The inhibitory effect of 1 was about 5200 times more potent than that of disodium cromoglycate (DSCG), which is a well-known antiallergic drug ($IC_{50}$ = 262 $\mu$M). IgE binding is a key event in Type I immediate hypersensitivity, and histamine release from mast cells and basophils relates the allergic reaction.[8] Effective inhibitors of histamine release from rat mast cells are used in the treatment of allergy and asthma. From these results, it is suggested that 1 and 2 are hopeful for an antiallergic drug. Detailed clarification of the pharmacological properties of 1 and 2 is in progress.

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(5) $^1H$ NMR (400 MHz, pyridine-$d_5$) $\delta$ 4.29 (H1), 4.46 (H2), 4.64 (H3), 2.85 (H4), 2.36 (H4), 2.60 (H5), 3.92 (H6), 5.15 (H7), 2.12 (H8), 2.01 (H9), 2.98 (H11), 2.21 (H11), 1.46 (H12), 1.21 (H12), 3.54 (H14), 2.73 (d, $J$ = 10.3 Hz, H16), 1.71 (dd, $J$ = 10.3, 10.3 Hz, H17), 1.22 (s, H18), 1.49 (s, H19), 2.63 (H20), 1.10 (d, $J$ = 5.9 Hz, H21), 2.10 (H22), 1.33 (dd, $J$ = 12.5, 12.5 Hz, H22), 1.98 (dd, $J$ = 13.9, 5.1 Hz, H24), 1.60 (dd, $J$ = 13.9, 6.6 Hz, H24), 2.21 (H25), 1.06 (d, $J$ = 8.1 Hz, H26), 1.04 (d, $J$ = 8.1 Hz, H27); $^{13}C$ NMR (100 MHz, pyridine-$d_5$) $\delta$ 77.0 (d, C1), 76.2 (d, C2), 70.6 (d, C3), 26.3 (t, C4), 42.0 (d, C5), 75.0 (d, C6), 75.0 (d, C7), 39.4 (d, C8), 48.3 (d, C9), 43.5 (s, C10), 25.0 (t, C11), 39.1 (t, C12), 38.1 (s, C13), 52.0 (d, C14), 217.6 (s, C15), 62.8 (d, C16), 58.0 (d, C17), 20.0 (q, C18), 10.0 (q, C19), 34.7 (d, C20), 20.8 (q, C21), 52.4 (t, C22), 82.2 (s, C23), 52.0 (t, C24), 25.0 (d, C25), 24.9 (q, C26), 25.3 (q, C27).

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## On the Mechanism of the Lewis Acid Mediated Cleavage of Chiral Acetals

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*Summary:* The $TiCl_4$-promoted cleavage of acetals has been shown to proceed by different mechanisms depending on the structure of the acetal, making it difficult to draw firm conclusions about the mechanism of related acetals based on model studies.

We have initiated a program to study methods for the asymmetric addition of nucleophiles to carbonyl deriva-

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