

PREGNA-1,4,20-TRIENE-3-ONE,  
A NOVEL MARINE STEROID FROM THE SEA RASPBERRY GERSEMIA RUBIFORMIS

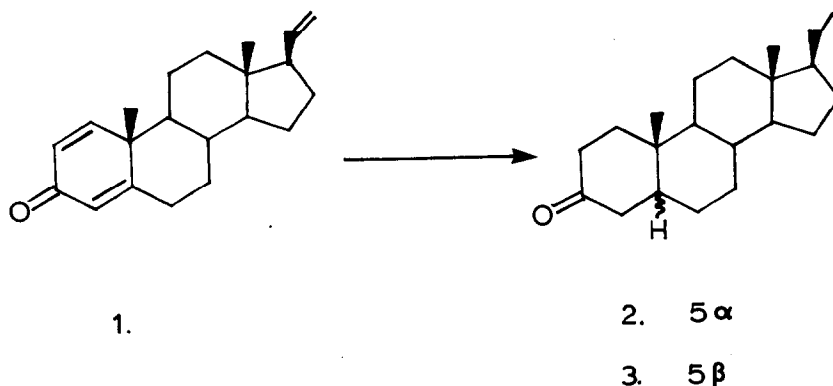
John F. Kingston, Brian Gregory, and Alex G. Fallis  
Department of Chemistry, Memorial University of Newfoundland,  
St. John's, Newfoundland, Canada, A1C 5S7

(Received in USA 27 July 1977; received in UK for publication 13 October 1977)

As part of our investigation of marine natural products from organisms inhabiting the cold waters off Newfoundland and Labrador we are examining a number of marine invertebrates including several soft corals of the order Alcyonacea (phylum Coelenterata, class Anthozoa, subclass Octocorallia). Warm water species of this order have yielded a number of novel natural products including sesquiterpenes,<sup>1</sup> cembranolide diterpenes,<sup>2</sup> and polyhydroxylated steroids.<sup>3</sup> We now wish to report the isolation of an unusual C<sub>21</sub> steroid with a vinyl side chain, 1, from Gersemia rubiformis (Pallas), an Alcyonacean coral known locally as the sea raspberry. In addition, we report its synthesis from progesterone.

Only two other examples of C<sub>21</sub> steroids from marine sources are known. A C<sub>21</sub> sterol has been reported as the aglycone from several starfish saponins,<sup>4</sup> and recently the first free pregnane derived sterols were isolated from the sponge Haliclona rubens.<sup>5</sup>

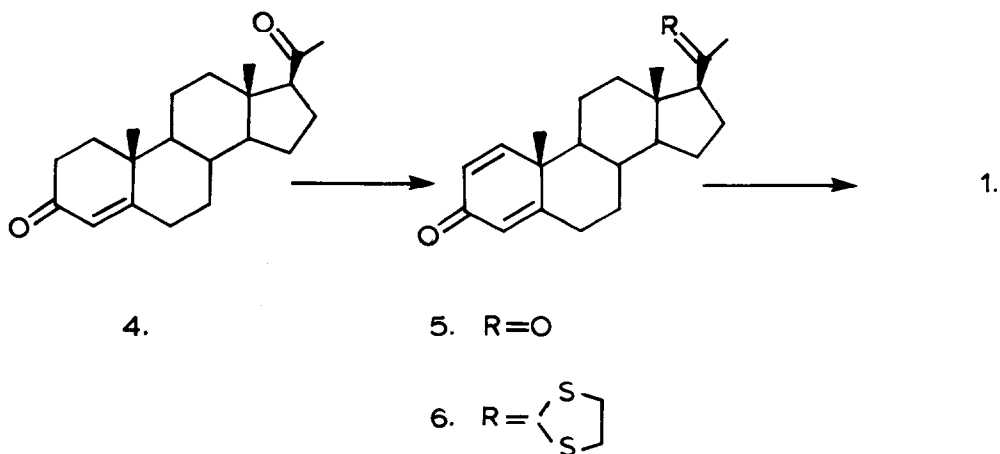
Specimens of Gersemia rubiformis were collected at Admiral's Cove, Cape Broyle, south of St. John's. The acetone extract of the freeze-dried animals was fractionated by high speed liquid chromatography on silica gel using a



stepped hexane:ether gradient. A fraction which eluted with 10% ether was subjected to preparative thin layer chromatography on alumina to afford the crystalline ketone 1, (m.p. 166-167°, from MeOH) in 0.05% yield based on the dry weight of the animal.

Hydrogenation of 1 over 5% Pd/C in ethyl acetate afforded a 1:2 mixture of 5 $\alpha$  and 5 $\beta$ -pregnane-3-one (2 and 3), which were identified by comparison with authentic samples.<sup>6</sup> This established the gross structural features of 1 and, with the data below, indicated that stereochemistry was 8 $\beta$ , 9 $\alpha$ , 14 $\alpha$ , 17 $\beta$ .

High resolution mass spectrometry established the molecular formula of 1 to be C<sub>21</sub>H<sub>28</sub>O (m/e 296.214  $\pm$  .001; calc 296.214). The infrared spectrum (CHCl<sub>3</sub>) displayed a strong conjugated ketone absorption ( $\lambda_{\max}$  1660 cm<sup>-1</sup>), as well as C=C stretching bands ( $\lambda_{\max}$  1615, 1595 cm<sup>-1</sup>). The ultraviolet spectrum ( $\lambda_{\max}$  (MeOH) 244 nm,  $\epsilon$  = 15,500) suggested the presence of a cross conjugated dienone.<sup>7</sup> Further evidence for this system was provided by the <sup>1</sup>H n.m.r. spectrum which showed signals for three olefinic protons at low field in a pattern characteristic of steroidal  $\Delta^{1,4}$ -3-ones.<sup>8</sup> A doublet at  $\delta$  6.93 (1H, J = 10Hz) and a doublet of doublets at  $\delta$  6.10 (1H, J = 10,2Hz) were assigned to the C-1 and C-2 protons respectively. The proton at C-4, coupled to the C-6 methylene as well as the C-2 proton, appeared as a compact multiplet at  $\delta$  5.97. The presence of the vinyl group was demonstrated by a complex ABC pattern of signals at approximately  $\delta$  4.8 (m, 1H),  $\delta$  5.0 (bs, 1H) and  $\delta$  5.6 (m, 1H). In addition, the spectrum displayed two three proton singlets at  $\delta$  0.63 and  $\delta$  1.20 assigned to the C-18 and C-19 methyl groups respectively. The base peak of the mass spectrum appeared at m/e 122 and is typical of a  $\Delta^{1,4}$ -3-keto steroid.<sup>9</sup> A common feature of the mass spectra of C-17 substituted steroids is the elimination of the side chain plus 42 mass units.<sup>10</sup> A major fragment at m/e 227 (M-69) therefore indicated a side chain of 27 mass units and is



consistent with the presence of the vinyl group in the assigned structure 1.

In order to verify this structural assignment a synthetic sample of 1 was prepared as follows. Treatment of progesterone (4) with DDQ in refluxing benzene afforded the known cross conjugated ketone 5.<sup>11</sup> The boron trifluoride etherate catalyzed reaction of 5 with ethanedithiol (1 eq.) gave predominantly the C-20 thioketal 6 (m.p. 221-223°, from MeOH/CHCl<sub>3</sub>). [Infrared (CHCl<sub>3</sub>):  $\lambda_{\max}$  1660, 1615, 1600 cm<sup>-1</sup>. Ultraviolet (MeOH):  $\lambda_{\max}$  245 nm,  $\epsilon = 13,500$ . <sup>1</sup>H n.m.r.: 18 Me,  $\delta$  0.87 (s, 3H); 19 Me,  $\delta$  1.22 (s, 3H); 21 Me,  $\delta$  1.83 (s, 3H); -SCH<sub>2</sub>CH<sub>2</sub>S-,  $\delta$  3.25 (m, 4H); H-C-4,  $\delta$  6.00 (m, 1H); H-C-2,  $\delta$  6.15 (dd, J = 10, 2Hz, 1H); H-C-1,  $\delta$  7.00 (d, J = 10Hz, 1H). Mass spectrum: m/e 383(4), 373(3), 327(3), 270(10), 121(19), 120(10), 119(100).] Desulfurization of 6 over Raney nickel was difficult to accomplish without concomitant reduction of one or more of the double bonds present in the molecule. However, catalyst which had first been rendered very inactive by prolonged (24 h.) refluxing in acetone effected a 20% conversion<sup>12</sup> of the thioketal to the expected<sup>13</sup>  $\Delta^{20}$  olefin 1. This synthetic material was indistinguishable from the natural product.

We have learned recently that Faulkner and Higgs have also isolated 1 as a minor component of a mixture of related C<sub>21</sub> derivatives from an unidentified Pacific soft coral.<sup>14</sup>

#### Acknowledgements

We are grateful to IBM Canada, Memorial University of Newfoundland, and the National Research Council of Canada for financial support of this research; H.M. Reiswig for identification of the sea raspberry; W.D. Jamieson for high resolution mass spectra; and to D.J. Faulkner for a preprint of their paper and fruitful discussion.

#### References and Footnotes

1. (a) J.C. Coll, S.J. Mitchell, and G.J. Stokie, Tetrahedron Letters, 1539 (1977);  
(b) Y.M. Sheikh, G. Singy, M. Kaisin, H. Eggert, C. Djerassi, B. Tursch, D. Dalozé, and J.C. Braekman, Tetrahedron, 32, 1171 (1976);  
(c) B. Tursch, M. Colin, D. Dalozé, D. Losman, and R. Karlsson, Bull. Soc. Chim. Belg., 84, 81 (1975);  
(d) M. Kaisin, Y.M. Skeikh, L.J. Durham, C. Djerassi, B. Tursch, D. Dalozé, J.C. Braekman, D. Losman, and R. Karlsson, Tetrahedron Letters, 2239 (1974);  
(e) B. Tursch, J.C. Braekman, D. Dalozé, P. Fritz, A. Kelecom, R. Karlsson, and D. Losman, Tetrahedron Letters, 747 (1974).
2. (a) Y. Kashman and A. Groweiss, Tetrahedron Letters, 1159 (1977);  
(b) M.G. Missakian, B.J. Burreson, and P.J. Scheuer, Tetrahedron, 31, 2513 (1975);

- (c) B. Tursch, J.C. Braekman, D. Dalozze, M. Herin, R. Karlsson, and D. Losman, Tetrahedron, 31, 129 (1975).
- 3.(a) B. Tursch, C. Hootele, M. Kaisin, D. Losman, and R. Karlsson, Steroids, 27, 137 (1976);  
(b) J.M. Moldowan, W.L. Tan, and C. Djerassi, Steroids, 26, 107 (1975).
- 4.(a) Y.M. Sheikh, B.M. Tursch, and C. Djerassi, J. Amer. Chem. Soc., 94, 3278 (1972);  
(b) Y. Shimizu, J. Amer. Chem. Soc., 94, 4051 (1972);  
(c) S. Ikegami, Y. Kamiya, and S. Tamura, Tetrahedron Letters, 1601 (1972).
5. J.A. Ballantine and K. Williams, Tetrahedron Letters, 1547 (1977).
6. (a) Authentic samples obtained from Steraloids Inc.:  
(b) R.E. Marker and E.J. Lawson, J. Amer. Chem. Soc., 60, 2438 (1938).
7. A.I. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products", Pergamon Press, Oxford, 1964, p. 64.
8. R.C. Tweit and C.M. Kagawa, J. Med. Chem., 7, 524 (1964).
- 9.(a) H. Budzikiewicz, "Biochemical Applications of Mass Spectrometry", Wiley-Interscience, New York, 1972, C.R. Waller, ed., p. 269;  
(b) K. Schubert, G. Kauffman, and H. Budzikiewicz, Biochim. Biophys. Acta, 176, 170 (1969);  
(c) R. Tschesche, I. Morner, and G. Snatzke, Justus Leibigs Ann. Chem., 670, 103 (1963).
10. H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry", Holden-Day Inc., San Francisco, 1964, Vol. 2, p. 94.
- 11.(a) T. Kubota, K. Yoshida, F. Hayashi, and K. Takeda, Chem. Pharm. Bull., 13, 50 (1965);  
(b) F. Sondheimer, M. Velasco, and G. Rosenkranz, J. Amer. Chem. Soc., 77, 5673 (1955).
12. In addition to the desired product, most of the material obtained from the reaction was unreacted thioketal (65%). No reduction products or  $\Delta^{17}$  isomer were detected under these conditions.
13. J. Fishman, M. Torigoe, and H. Guzik, J. Org. Chem., 28, 1443 (1963).
14. M.D. Higgs and D.J. Faulkner, Steroids, 30, 0000 (1977) in press.