Tetrahedron Letters,Vol.27,No.36,pp 4287-4290,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

# VENUSTATRIOL. A NEW, ANTI-VIRAL, TRITERPENE TETRACYCLIC ETHER FROM *LAURENCIA VENUSTA*

Shinichi Sakemi and Tatsuo Higa*[1],
Harbor Branch Foundation-SeaPharm Research Laboratories,
RR #1, Box 196, Fort Pierce, Florida 33450, USA,
and
Charles W. Jefford* and Gérald Bernardinelli,
Department of Organic Chemistry and Laboratory of Crystallography, University of Geneva,
1211 Geneva 4, Switzerland

*Abstract*. Venustatriol, a new tetracyclic ether derived from squalene, which has anti-viral activity, has been isolated from the red alga *Laurencia venusta*. Its structure and absolute configuration were determined by X-ray using the absolute structure parameter x. By comparison of their spectral properties, the absolute configuration of its congener, thyrsiferol, was deduced.

In our search for biologically active constituents of marine organisms living in Okinawan waters, we discovered that a crude extract of *Laurencia venusta* displayed significant activity against the vesicular stomatitis virus (VSV) and herpes simplex virus type 1 (HSV-1). Subsequent fractionation of the extract using assays of activity as a guide led to the separation of the active components which were identified as thyrsiferol (1), thyrsiferyl-23 acetate (2), and a hitherto unknown, but structurally related metabolite, venustatriol (3).

We now describe the isolation of 3 and the elucidation of its structure. A fresh sample (1.2 kg) of the alga, collected on the coast at Onna, Okinawa, in April 1985, was extracted with acetone. Concentration of the extract gave an aqueous suspension which was further extracted with ethyl acetate. The resulting oily residue (3 g) was separated by chromatography over silica gel by eluting with heptane containing increasing amounts of acetone. Each fraction was assayed against VSV and HSV-1. The active fractions were combined, purified by chromatography over silica gel (chloroform-acetone, 8:1) and finally submitted to HPLC (Dynamax Si column, heptane-ethyl acetate). Thyrsiferol (1) and its 23-acetate (2) were obtained as colorless glasses (85 and 78 mg)[2,3] and venustatriol (3) as a white solid (9.7 mg).

The identification of 1 and 2 was straightforward, as they are known. Compound 3 is new, but fortunately has physical properties similar to those of 1, a congener also found in *Laurencia thyrsifera*,[4] and to those of dehydrothyrsiferol[5] and thyrsiferyl-23 acetate (2)[6] occurring in other species of *Laurencia*.

Venustatriol (3), mp 161.5° $[\alpha]_D^{20}$ +9.4° (c 3.2, $CHCl_3$), exhibited the following characteristic data. EIMS: m/z 588 (1.2), 586 ($M^+ - H_2O$, 1.1), 570 (0.7), 568 (0.7), 552 (0.9), 550 (0.9), 529 (1.1), 527 (1.1), 524 (2.8), 506 (6), 491 (6), 486 (6), 470 (2.8), 445 (3.8), 443

(3.7), 409 (5), 363 (10), 227 (78), 209 (75), 207 (25), 205 (26), 193 (11), 165 (10), 127 (42), 125 (100), 111 (31), 109 (40), and 107 (40). IR (KBr): 3360, 2960, 1445, 1370, 1173, 1118, 1095, 1083, and 1015 $cm^{-1}$. $^{1}$H-NMR ($CDCl_3$): $\delta$ 1.087 (3H, s), 1.135 (3H, s), 1.173 (3H, s), 1.180 (3H, s), 1.194 (3H, s), 1.267 (6H, s), 1.396 (3H, s), 3.044 (1H, dd, J=2.4, 11.2 Hz), 3.564 (1H, dd, J=7.3, 11.0 Hz), 3.591 (1H, dd, J=1.1, 10.1 Hz), 3.708 (1H, dd, J=2.8, 12.5 Hz), 3.825 (1H, dd, J=7.3, 7.3 Hz), and 3.890 (1H, dd, J=4.1, 12.2 Hz). $^{13}$C-NMR ($CDCl_3$): $\delta$ 20.02, 20.53, 21.12, 21,46, 22,98 (2C), 23.66, 23.81, 25.50, 25.90, 26.91, 27.55, 28.20, 30.98, 31.14, 33.10, 37.02, 38.55, 58.98, 71.88, 72.07, 73.23, 74.37, 74.91, 75.68, 76.30, 77.18, 77.55, 84.36, and 86.50.

As thyrsiferol (1) was characterized as its C-18 acetate, the corresponding acetate of 3 was prepared so as to facilitate comparison. Venustatriol-18 acetate: $^{1}$H-NMR ($CDCl_3$): $\delta$ 1.062 (3H, s), 1.107 (3H, s), 1.154 (3H, s), 1.192 (3H, s), 1.215 (6H, s), 1.266 (3H, s), 1.396 (3H, s), 2.070 (3H, s), 3.036 (1H, dd, J=2.2, 11.5 Hz), 3.550 (1H, dd, J=7.4, 11.1 Hz), 3.672 (1H, dd, J=2.9, 12.8 Hz), 3.761 (1H, dd, J=7.5, 7.5 Hz), 3.892 (1H, dd, J=4.0, 12.3 Hz), and 4.956 (1H, dd, J=2.3, 9.7 Hz). $^{13}$C-NMR ($CDCl_3$): 20.05, 20.61, 21.06, 21.24, 21.40, 22.81, 23.02 (2C), 23.66, 24.09, 24.40, 26.26, 27.56, 28.23, 31.00, 32.25, 34.14, 37.05, 38.49, 59.01, 70.94, 71.94, 73.20, 74.37, 74.95, 75.71, 76.26, 78.26, 84.29, 84.99, 86.53, and 170.93.

The molecular formula of 3, $C_{30}H_{53}BrO_7$, is inferred from the ($M^+$-$H_2O$) doublet (588, 586). The number of carbon atoms is confirmed by the $^{13}$C-NMR signals. Careful inspection of the foregoing NMR data with that of 1 and 2 and their acetates reveals that 1 and 3 possess the same array of six-membered rings. However, there is an unspecified difference in configuration of the substituents attached to the tetrahydrofuran ring. Consequently, in order to dispel all structural ambiguity, a suitable crystal of 3 was grown from a solution of heptane and dichloromethane. Its absolute configuration was determined by X-ray[7] using the absolute structure parameter x[8] (Fig. 1). The result establishes the configuration of the ten chiral centers of venustatriol, but also permits the correct assignment of configuration to thyrsiferol. Both molecules share the same configuration for the asymmetric centers situated on rings A, B, and C, namely C3R, C6S, C7R, C10S, C11R, C14R, and C15S (Fig. 2). Furthermore, they also possess the same spatial disposition as rings A and B exist as chairs, whereas the C ring in both molecules is obliged to adopt a boat conformation to avoid an otherwise unfavorable 1,3 diaxial interaction between substituents in the alternative chair conformation. The essential difference resides in the tetrahydrofuran substituent pattern; the configurations of the C18 and C19 atoms in thyrsiferol are just the opposite of those in venustatriol, while those at the C22 position remain the same (Fig. 2).[9]

As far as we are aware, venustatriol and thyrsiferol are the only tetracyclic ethers of triterpenoid origin so far reported.[10] Their occurrence points to a two-stage process for their biogenesis from a common squalene penta-epoxide precursor. The first step is the same for both products. It undoubtedly entails attack by brominium ion at the C2-C3 double bond, causing the concerted cyclization of three epoxides identically configured at C6-C7, C10-C11, and C14-C15, thereby producing rings A, B, and C. The furan ring is formed in a second step, but in two ways; either by protonation of the C18-C19 or the C22-C23 epoxide.

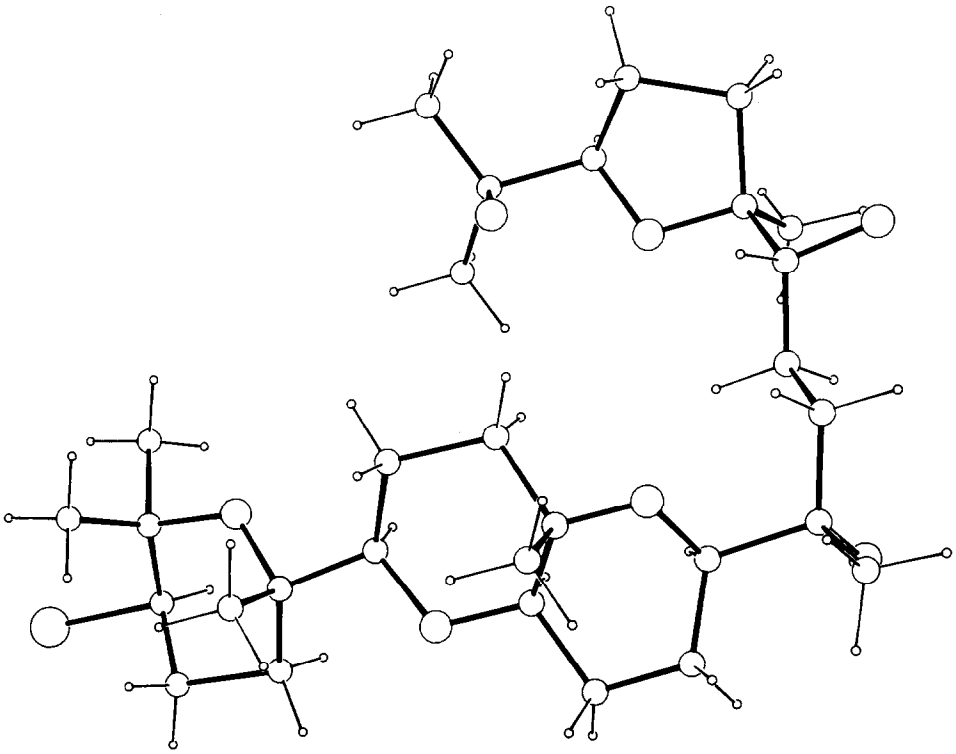
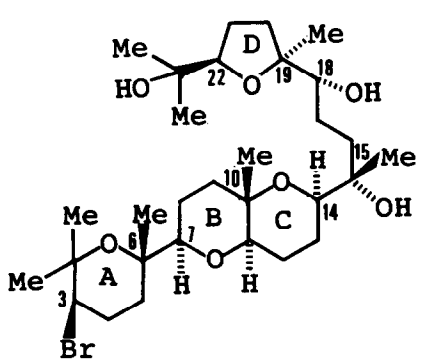
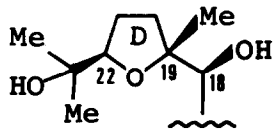


*Fig. 1.* X-ray structure showing the absolute configuration of venustatriol (3)




3




stereochemistry of rings A, B, C, and C15 the same as in 3

1

*Fig. 2.* Configurations of venustatriol (3) and thyrsiferol (1)

*Acknowledgments*. We thank Dr. S. Cross for the anti-viral tests and Drs. M.H.G. Munro and J.W. Blunt for valuable discussions.

## REFERENCES AND NOTES

1. On leave from the Department of Marine Sciences, University of the Ryukyus, Nishihara, Okinawa 903, Japan.
2. Compound 1: mp 133-136°, $[\alpha]_D^{20}$ +6.4° (c 5, $CHCl_3$) (lit.[5] $[\alpha]_D$ +6.8° (c 0.16, $CHCl_3$)). The $^1$H-NMR and $^{13}$C-NMR spectra of 1 were practically identical with those of authentic thyrsiferol, which were kindly provided by Prof. Munro.
3. Compound 2: mp 115-116° (lit.[6] mp 118-119°) $[\alpha]_D^{20}$ +2.64° (c 5.7, $CHCl_3$) (lit.[6] $[\alpha]_D$ +1.99). It was identified by comparison of its spectral data with those reported for 2. Moreover, its saponification gave a sample identical with 1.
4. J.W. Blunt, M.P. Hartshorn, T.J. McLennan, M.H.G. Munro, W.T. Robinson, and S.C. Yorke, *Tetrahedron Lett.* 69 (1978).
5. A.G. González, J.M. Arteaga, J.J. Fernandez, J.D. Martín, M. Norte, and J.Z. Ruano, *Tetrahedron* 40, 2751 (1984).
6. T. Suzuki, M. Suzuki, A. Furusaki, T. Matsumoto, A. Kato, Y. Imanaka, and E. Kurosawa, *Tetrahedron Lett.* 26, 1329 (1985).
7. Crystal data: The crystals are orthorhombic; space group $P2_12_12_1$, a=6.7074(15), b=20.346(4), c=23.771(3) Å; Z=4; $d_c$=1.240 g·cm$^{-3}$; $\mu$=1.292 mm$^{-1}$. Data were collected on a Philips PW 1100 diffractometer (MoK$\alpha$). The structure was solved by direct methods (MULTAN-80) and refined by full-matrix least-squares analysis. The absolute configuration was determined by least-squares refinement of the absolute structure parameter x[8] (x=0.08(7)). The final R-factor, based on 1022 reflections was 0.087. Complete crystallographic data will be published in the full paper.
8. G. Bernardinelli and H.D. Flack, *Acta Crystallogr., Sect. A* 41, 500 (1985).
9. In previous papers,[4-6] the relative configurations at the C14 and C15 positions of thyrsiferol have been mistranscribed from the X-ray view[4] of thyrsiferyl-18 acetate.
10. Recent examples of non-triterpenoid cyclic polyethers of marine origin are brevetoxin (Y. Shimizu, H.N. Chou, H. Bando, G. Van Duyne, and J.C. Clardy, *J. Am. Chem. Soc.* 108, 514 (1986) and norhalichondrin A (D. Uemura, K. Takahashi, T. Yamamoto, C. Katayama, J. Tanaka, Y. Okumura and Y. Hirata, *J. Am. Chem. Soc.* 107, 4796 (1985).

(Received in Germany 3 June 1986)