Tetrahedron Letters, Vol.31, No.34, pp 4895-4898, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

STRUCTURE ELUCIDATION OF LAUREOXANYNE, A NEW NONISOPRENOID C_{15} ENYNE, USING LACTOPEROXIDASE

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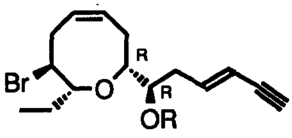
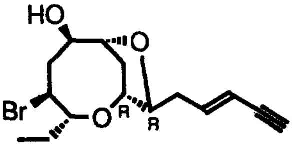
Abstract: The enzymatic structure elucidation of a minor bicyclic nonisoprenoid C_{15} enyne, laureoxanyne, isolated from the red alga *Laurencia nipponica*, is described.

Cyclic nonisoprenoid C_{15} enynes from *Laurencia nipponica* can be divided into two groups; those possessing (6*R*,7*R*) configuration, e.g. laurencin (1)[1] and laurefucin (2)[2] and those possessing (6*S*,7*S*) configuration, e.g. laureatin (3),[3] isolaureatin (4),[3] and laurallene (5).[4] These acetylenes in each group are isolated individually from the sibling species growing in close proximity at Oshoro Bay in Hokkaido. From one of the populations, we isolated a new minor bicyclic bromo-ether, designated as laureoxanyne (6), along with the co-occurring major constituents 1 and 2. We succeeded in biogenetically relating the new acetylene to the (6*R*,7*R*)-group. We describe herein the structure elucidation of laureoxanyne (6) on the basis of spectral data and an enzymatic reaction. This compound is valuable to reveal a biosynthetic pathway to 2 and notoryne (7)[5] from 1 or deacetyllaurencin (8).[1]



1 R=Ac
8 R=H



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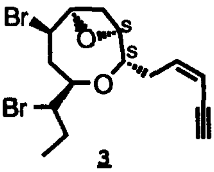

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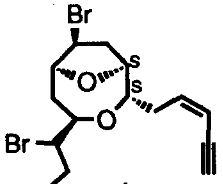

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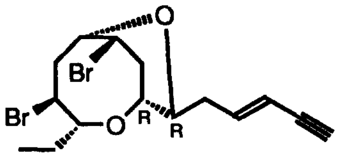

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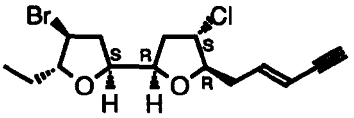

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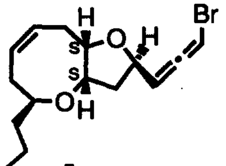

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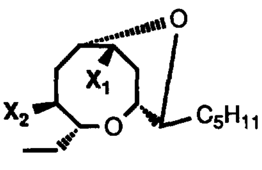
Ether soluble neutral oil (18 g), obtained from the methanol extracts of *L. nipponica* (6 Kg, wet weight), was repeatedly separated on silica gel and purified twice by HPLC to give laureoxanyne [(6), (2 mg)] as a colorless oil.

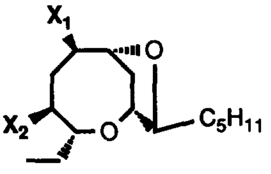
Laureoxanyne (6), $C_{15}H_{20}O_2Br_2$, displayed the IR bands indicating the presence of a characteristic terminal acetylene conjugated to (*E*)-double bond, and ether functionalities, but no carbonyl and hydroxyl groups.[6] The UV spectrum [(EtOH) 224 (ε 16,900) and 232 nm (infl. ε 13,500)] supported the presence of a conjugated enyne unit. The ^{1}H NMR spectrum exhibited clearly-resolved signals which involved an acetylenic proton at δ 2.85, two olefinic protons at δ 5.62 and 6.29, six protons assignable to two bromomethynes and four oxy-methynes in the region of δ 3.09-4.19. The five degrees of unsaturation are thus accounted for one double bond, one triple bond, and two ether rings. Homo-nuclear decoupling experiments led to the complete assignment of all protons for the straight carbon skeleton.

The structure of 6 could not be elucidated by usual degradation methods due to its small quantity. Since our preliminary studies on (9*E* or 9*Z*)- and (12*E*)-pentadecene-6,7-diols revealed that they were converted to the corresponding endo-cyclic bromooxolanes and an oxocane, respectively, with positive bromine generated from bromide in the presence of lactoperoxidase and hydrogen peroxide,[7] we attempted to determine its structure by enzymatic conversion of the known deacetyllaurencin (8) to 6. Deacetyllaurencin [(8), 205 mg] dissolved in DMSO (2 ml) was added to 200 ml of phosphate buffer (pH 5.5) containing NaBr (0.3 mM). Each solution of lactoperoxidase (64 nM) and hydrogen peroxide (0.8 mM) was divided into nine portions and the aliquots were added to the reaction mixture in every 10 min in the dark at room temperature. After stirring for 24 h, the mixture was extracted with ethyl acetate, whose extracts were purified on silica gel followed by HPLC to afford 6 (8.2 mg), 2 (13.9 mg), bromohydrins (13.7 mg), and the recovered substrate (124.6 mg). The compound 6 thus obtained was completely identical with natural laureoxanyne in all respects including optical rotation, $[\alpha]_D^{24}$ +19.3° (*c* 0.5, $CHCl_3$). This result indicates that the compound (6) must possess laurencin-type configurations at C-6, C-7, C-12, and C-13. The additional stereochemistry at C-9 and C-10 have been elucidated chemically and spectroscopically as follows.

Hydrogenation of 6 over Pd-C in ethanol gave hexahydrolaureoxanyne (9), $C_{15}H_{26}O_2Br_2$,[8] which was apparently different from the compound 10 derived from hexahydrolaurefucin (11)[2] by bromination with $SOBr_2$. Furthermore, complete debromination of 9 with Ra-Ni in ethanol afforded 12,[9] while 10 gave 13.[10] This result revealed that 6 has a six-membered ether ring between C-10 and C-6. The configuration of the bromine atom at C-9



9 $X_1=X_2=Br$
12 $X_1=X_2=H$



10 $X_1=X_2=Br$
11 $X_1=OH$, $X_2=Br$
13 $X_1=X_2=H$

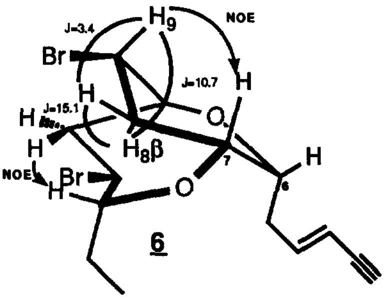
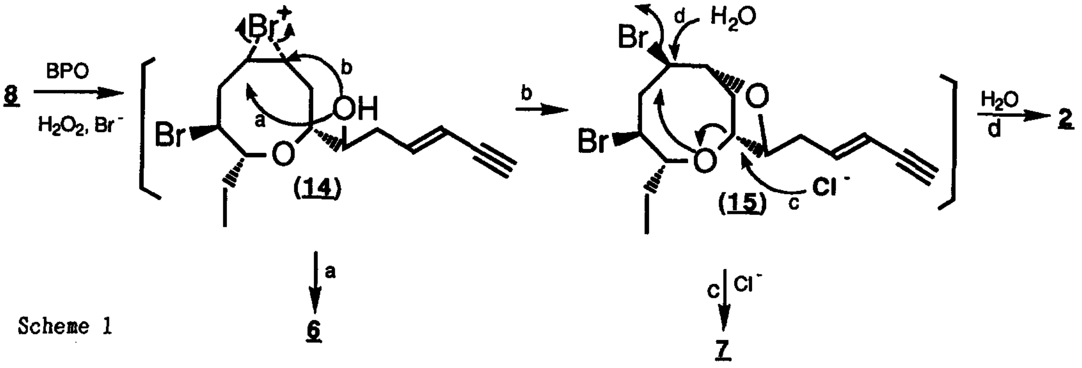


Fig. 1

was determined by NOE experiments: irradiation of H-9 at δ 4.19 showed a large NOE on H-7 at δ 3.25, whereas a small enhancement of H-8α proton signal at δ 2.03 was observed. The coupling constant between H-8β and H-9 (10.7 Hz) showed the diaxial-orientation on the chair-like oxane ring (Fig. 1). Thus, the structure of laureoxanyne was represented as 6.

The structure of laureoxanyne (6) and enzymatic reaction mechanism initiated by lactoperoxidase strongly suggested the presence of biosynthetic intermediates 14 and 15 as shown in Scheme 1.



Scheme 1

Although the intermediate 15 could not be isolated from the alga, spontaneous conversion of 10 into hexahydrolaurefucin (11) in aqueous solvents supported this proposed biosynthetic pathway to laurefucin (2). Notoryne (7) having the (6*R*,7*S*) configuration might be a rearranged product which may be caused by the attack of chloride ion at C-7 of 15 with inversion of configuration. The enzymatic structure determination based on the biosynthesis of nonisoprenoid C_{15} enyne described here demonstrates to be a useful method for even minor natural metabolites.

References and Notes

1. T. Irie, M. Suzuki, and T. Masamune, *Tetrahedron Lett.*, 1980, 21, 1471.
2. A. Furusaki, E. Kurosawa, A. Fukuzawa, and T. Irie, *Tetrahedron Lett.*, 1973, 4579.
3. T. Irie, M. Izawa, and E. Kurosawa, *Tetrahedron*, 1970, 26, 851.
4. A. Fukuzawa and E. Kurosawa, *Tetrahedron Lett.*, 1979, 2797.
5. H. Kikuchi, Ph.D. Thesis, Hokkaido University, Japan, 1983.
6. 6: $[\alpha]_D^{24}+20.9°$ (c 0.31, $CHCl_3$); FI-MS, m/z 390, 392 and 394 (M^+); HRFIMS, m/z 392.9863 [Calcd for $C_{15}H_{21}O_2{}^{79}Br^{81}Br$: 392.9888]; IR (neat), 3292, 2104, 1640, 1296, 1262, 1198, 1028, and 960 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 2.85, (1H, d, J=2.0 Hz, H-1), 5.62 (1H, ddd, J=16.1, 2.0, 1.4, H-3), 6.29 (1H, dt, J=16.1, 8.5, H-4), 2.37 (1H, dddd, J=14.0, 8.5, 6.3, 1.4, H-5), 2.39 (1H, dddd, J=14.0, 8.5, 6.3, 1.4, H-5'), 3.09 (1H, dt, J=4.4, 6.3, H-6), 3.25 (1H, dt, J=7.8, 4.4, H-7), 2.03 (1H, ddd, J=15.1, 7.8, 3.4, H-8α), 2.23 (1H, ddd, J=15.1, 10.7, 4.4, H-8β), 4.19 (1H, dt, J=10.7, 3.4, H-9), 4.13 (1H, ddd, J=9.8, 8.3, 3.4, H-10), 2.69 (1H, dt, J=13.2, 8.3, H-11α), 2.35 (1H, dt, J=13.2, 9.8, H-11β), 3.88 (1H, dt, J=9.8, 8.3, H-12), 4.02 (1H, dt, J=8.3, 3.9, H-13), 1.53 (1H, ddq, J=14.0, 3.9, 7.5, H-14), 1.79 (1H, ddq, J=14.0, 3.9, 7.5, H-14'), and 1.01 (3H, t, J=7.5, H-15).
7. The bromoperoxidase (BPO) activity was detected in crude extract from the alga and purification of the enzyme is in progress. Commercially available lactoperoxidase (LPO) was employed for our synthetic studies because of its similar nature to BPO. Cf. A. Fukuzawa, Mya Aye, A. Murai, M. Nakamura, and M. Tamura, Abstract of 31st Symposium on the Chemistry of Natural Product (Japan), 1989, p. 300.
8. 9: $[\alpha]_D^{20}+27.1°$ (c 0.27, $CDCl_3$); HREIMS, m/z 317.1125 (M^+-Br); IR (neat), 1294, 1262, 1074, and 1018 cm^{-1}; ^1H NMR ($CDCl_3$), δ 0.90 (3H, br.t, J=7.5 Hz), 1.01 (3H, t, J=7.5), 1.32 (3H, m), 1.57 (6H, m), 1.79 (1H, ddq, J=3.9, 14.0, 7.5), 2.03 (1H, ddd, J=15.1, 6.8, 3.4), 2.23 (1H, ddd, J=15.1, 6.7, 4.8), 2.34 (1H, dt, J=13.0, 9.8), 2.67 (1H, dt, J=13.2, 8.3), 3.01 (1H, dt, J=4.4, 6.3), 3.21 (1H, dt, J=6.8, 4.4), 3.87 (1H, dt, J=9.8, 8.3), 4.02 (1H, dt, J=8.3, 3.9), 4.13 (1H, ddd, J=9.8, 8.3, 3.4), and 4.20 (1H, dt, J=10.7, 3.42).
9. 12: $C_{15}H_{28}O_2$, FD-MS, m/z 242 (M^++2); ^1H NMR, δ 3.95 (1H, qui, J=6.4 Hz), 3.87 (1H, qui, J=6.4), and 2.93 (2H, m).
10. 13: $C_{15}H_{28}O_2$, FD-MS, m/z 242 (M^++2); ^1H NMR, δ 4.34 (1H, ddd, J=11.2, 4.3, 2.4 Hz), 3.98 (1H, m), 3.71 (1H, dt, J=2.4, 7.2), and 3.38 (1H, br.m).

(Received in Japan 30 May 1990)