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BRASUDOL AND ISOBRASUDOL: TWO BROMOSESQUITERPENES FROM A SEA HARE (APLYSIA BRASILIANA)

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The structure and stereochemistry of two closely related new feeding deterrents, isolated from the digestive glands of the mollusk Aplysia brasiliana, are reported. These compounds, which proved to be brominated eudesmanes, have the same absolute configuration as natural β-eudesmol.

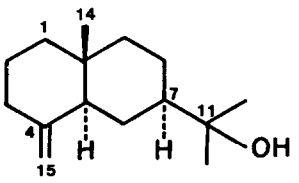
We have previously reported that the marine mollusk Aplysia brasiliana is distasteful to fish and rejected by sharks.[1] Several fish antifeedants have now been isolated from this mollusk in our laboratory.[2] One of these substances is the brominated sesquiterpene brasudol, which was shown to be a potent feeding deterrent.[3] Also isolated as a minor constituent of A. brasiliana was the isomeric isobrasudol. We report here experiments defining the structures and absolute configurations of these isomeric sesquiterpene bromo-alcohols.

Two A.brasiliana digestive glands[4] (15.4 g wet weight) were ground in a Waring blender with an ether/methylene chloride (1:1 vol/vol) mixture. Fractionation of the extract (1.7 g) by gradient column chromatography [silica gel, 50% (vol/vol) hexane/benzene to 50% (vol/vol) hexane/ethyl acetate] followed by thin-layer chromatography afforded 232 mg (14%) of brasudol (mp 105-106°C; $[\alpha]_D^{26}$ + 16.5°; Anal. Calcd for $C_{15}H_{25}BrO$: C, 59.84; H, 8.30; Br, 26.54. Found: C, 59.53; H, 8.57; Br, 26.28) and 57 mg (3%) of isobrasudol (mp 105-107°C; $[\alpha]_D^{26}$+ 10.3°; m/e 283.1029, 285.1031; Calcd for $C_{15}H_{24}Br$ (M^+-OH), 283.1062, 285.1042). The infrared spectrum of brasudol revealed the presence of a tertiary hydroxyl group [3585 (s), 3460 (v br, m), 1162 (m) cm^{-1}] and a disubstituted terminal double bond [1651 (s), 892 (s) cm^{-1}].

Observation of a six proton singlet at 1.21 δ in the 270 MHz pmr spectrum[5] of brasudol indicated the presence of a 2-(2-hydroxypropyl) substituent. A tertiary methyl substituent (0.82 δ, 3 H), the protons of a disubstituted terminal double bond (4.57 δ, 1 H; 4.80 δ, 1 H), and a low field multiplet (4.06 δ, 1 H, width = 15 Hz) indicative of an axial proton on a bromine-bearing carbon adjacent to a methylene group[6] were also observed. Carbon skeleton chromatography[7] and consideration of the data already presented suggested that brasudol might be a brominated derivative of β-eudesmol (1). This was confirmed by lithium/ammonia reduction of brasudol to afford a product identical in all respects (ir, pmr, $[\alpha]_D$) to an authentic sample of natural β-eudesmol.[8]

Comparison of the cmr spectra of brasudol and 1 (Table 1) and consideration of the possible biosynthetic origin of this halogenated sesquiterpene[9] allowed tentative assignment of structure

2 to brasudol. The upfield position of the angular methyl carbon in brasudol (11.9 δ) compared to that in 1 (16.1 δ) requires the bromine atom to be γ and gauche (i.e. equatorial) to this methyl group.[10] Confirmation of this assignment was provided by the following transformations:



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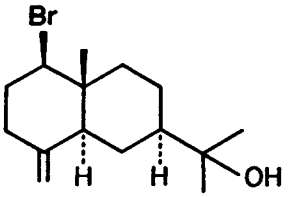

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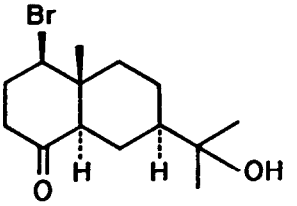
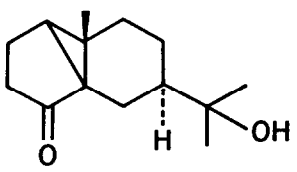


3

ozonolysis of 2 in methylene chloride followed by reductive workup afforded bromoketone[11] 3 which gave a halogen-free hydroxyketone[12] (4) when heated at reflux with lithium carbonate in DMF. This dehydrobromination product displayed no olefinic protons and no protons above 0.83 δ in its pmr spectrum. In addition, the carbonyl absorption at 1720 cm^{-1} corresponds to that observed for several bicyclo[3.1.0]hexanones,[13] but is inconsistent with the formulation of 4 as a bicyclo[4.1.0]heptanone analogous to 5 (ir 1670 cm^{-1}).[14] The single-frequency off-resonance decoupled cmr spectrum of 4 was consistent with the assigned structure.



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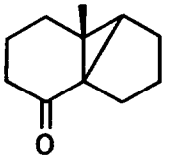

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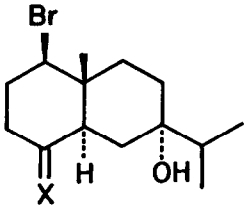


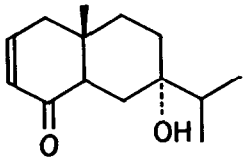
6 X = CH_2

7 X = O

The infrared spectrum of isobrasudol again indicated the presence of a tertiary hydroxyl group [3600 (m), 3480 (v br, m), 1135 (m) cm^{-1}] and a disubstituted terminal double bond [1650 (s), 895 (s) cm^{-1}]. Its 100 MHz pmr spectrum displayed an apparent nine proton triplet in the methyl region. This pattern could most readily be understood as the fortuitous overlap of a tertiary methyl singlet and the doublets arising from two diastereotopic methyl groups of an isopropyl substituent. Thus, addition of 0.1 equivalents of $Eu(fod)_3$ shift reagent resolved this apparent triplet into a singlet and two doublets. A low field multiplet (3.95-4.18 δ, 1 H) indicative of an axial proton on a bromine-bearing carbon,[6] and two singlets (4.58 δ, 1 H; 4.81 δ, 1 H) corresponding to the protons of a disubstituted terminal double bond formed a pattern strikingly similar to that observed for 2. In addition, the facile loss of a C_3H_7 unit [m/e 257.0521, 259.0506] in the high resolution mass spectrum of isobrasudol suggested the presence of an isopropyl group attached to a carbon atom bearing oxygen. Carbon skeleton chromatography[7] and consideration of the spectral data permitted formulation of isobrasudol as 6. The positions of the bromine and hydroxy substituents were confirmed by chemical degradation. Ozonolysis of 6

followed by reductive workup afforded bromoketone 7[15] which gave two halogen-free hydroxy ketones 8[16] and 9[17] when heated at reflux with lithium carbonate in DMF.

The orientation of the hydroxyl substituent in isobrasudol was determined by a study of the lanthanide induced shifts in the cmr spectrum of 6 using $Eu(fod)_3$. The downfield shifts of the carbon resonances were consistent with an axial hydroxyl group [Slope ($\Delta\delta$ vs [LSR]/[S])= -3.0] and not with an equatorial hydroxyl substituent [plot ($\Delta\delta$ vs [LSR]/[S])=scattering of points]. Finally, the absolute configuration of isobrasudol was rigorously established by conversion [a) Li/liq NH_3; b)p-TsOH/PhH/Δ, 1h][18a] to (+)-δ-selinene (10) ($[a]_D^{25}$ + 194°) which was identical in all respects (ir, pmr, ms), except the sign of its optical rotation, to an authentic sample of (-)-δ-selinene.



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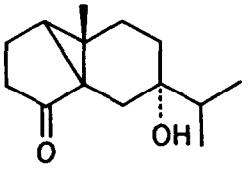

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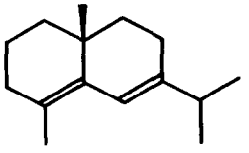

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Interestingly, we have also observed these two compounds in the red alga Chondria cnicophylla upon which A. brasiliana feed. It is noteworthy that brasudol and isobrasudol have absolute configurations corresponding to that of β-eudesmol from terrestrial sources,[8] and opposite from that of similar halogenated sesquiterpenes recently isolated from Laurencia sp.[18]

TABLE 1

cmpd	c1	c2	c3	c4	c5	c6	c7	c8	c9	c10	c11	c12	c13	c14	c15
1	41.0$^+$	23.3	36.7	150.9	49.6*	24.8	49.3*	22.2	41.7$^+$	35.7	72.7	27.0	27.0	16.1	105.2
2	67.8	34.9	37.1	147.5	49.5	25.5	48.9	22.4	39.6	41.0	72.5	26.9*	27.2*	11.9	107.6
3	63.2	33.1	41.4	207.0†	56.7	21.8$^+$	47.8	22.0$^+$	39.7	43.0	72.3	27.2*	26.8*	12.2	----
6	67.0	34.7	36.9	146.7	45.7	34.9	73.9	31.9	36.6	40.8	28.8	15.9	15.9	11.9	107.8
7	62.6	33.0	41.2	207.9†	53.1	31.3	73.3	31.3	36.7	42.6	28.6	15.7	15.7	12.3	----

a) The cmr chemical shifts are in ppm down-field from TMS and are referenced with respect to internal $CDCl_3$. b) $^{+,*}$ assignments may be reversed. c) † Calcd from reflected peaks.

ACKNOWLEDGEMENTS

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3. A description of the bioassay employed to assess the activity of these compounds will be described elsewhere.

4. The A. brasiliana were obtained from the Gulf Specimen Co., Inc., Panacea, Florida.

5. The 270 MHz pmr spectra were obtained from the Southern New England High Field NMR Facility at Yale University, New Haven, Conn.

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8. A sample of natural β-eudesmol was generously provided by Professor J.A. Marshall, Northwestern University.

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11. mp 122.5-123.5°C; ir (CCl_4) 3600(m), 3500 (v br, w), 2960(s), 2858(s), 1720(s), 1468(m), 1443(m), 1386(m), and 1120(s) cm^{-1}; nmr ($CDCl_3$) δ 0.88 (s, 3 H), 1.20 (s, 6 H), 1.10-2.19 (m, 9 H), 2.19-2.66 (m, 4 H), 4.27 (m, 1 H); m/e (chemical ionization) 303, 301, 287, 285.

12. ir (CCl_4) 3650(m), 3500(v br, m), 2960(s), 2890(s), 1720(s), 1470(m), 1415(m), 1390(m), and 1130(m) cm^{-1}; nmr ($CDCl_3$) δ 1.06 (s, 3 H), 1.14 (s, 6 H), 0.83-1.37(m, 3 H), 1.37-2.46(m, 10 H); cmr ($CDCl_3$) δ 17.1, 18.2, 22.2, 22.4, 26.4, 27.2, 27.3, 35.6, 38.0, 39.0, 43.1, 43.8, 72.6, 209.2; m/e 222.1636.

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15. mp 127.0-128.5°C; ir (CCl_4) 3592(m), 3560-3300 (v br, m), 2952 (vs), 1725 (vs), 1460 (m), 1390 (m), 1360 (m), 1280 (m), 1235 (m), 1172 (m), 940 (m); nmr ($CDCl_3$) δ 0.85 (d, J=6 Hz, 3 H), 0.92 (d, J=6 Hz, 3 H), 0.94 (s, 3 H), 1.09-2.14 (m, 9 H), 2.15-2.30 (m, 4 H), 4.27 (m, 1 H); m/e (chemical ionization) 303, 301, 287, 285.

16. ir 3590 (s), 3470 (v br, m), 2945 (vs), 1687 (vs) cm^{-1}; nmr ($CDCl_3$) δ 0.90 (d, J=6 Hz, 3 H), 0.93 (d, J=6 Hz, 3 H), 0.97 (s, 3 H), 6.04 (dt, J=4, 10 Hz, 1 H), 6.80 (dt, J=2, 10 Hz, 1 H); UV (CH_3OH) 227nm (ε9600).

17. ir 3590 (m), 3475 (v br, s), 2940 (s), 1720 (s), 1700 (s), 1470 (m), 1390 (m), 1308 (m), 1260 (m), 950 (m) cm^{-1}; nmr ($CDCl_3$) δ 0.88 (d, J=6 Hz, 6 H), 1.13 (s, 3 H), 1.17-2.50 (m, 13 H); m/e 222.1622.

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