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Marine Natural Products: Isodactylyne, a Halogenated Acetylenic Ether from the Sea Hare *Aplysia dactylomela*[1]

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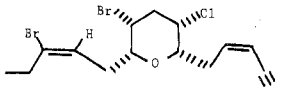
In a previous communication[2] we reported the structure of dactylyne (1), which was isolated from the sea hare *Aplysia dactylomela*. We report herein the isolation of an isomeric halogenated acetylenic ether, isodactylyne, from the same source and present evidence for assigning it the structure **2**.



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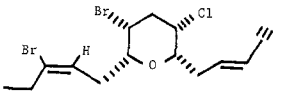

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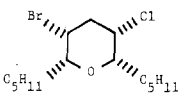
These two halogenated ethers are members of a group of similar nonterpenoid C-15 ethers elaborated by red algae.[3]

Isodactylyne, a colorless oil, $[\alpha]^{24}D$ −8.06°, was isolated by chromatography from the hexane extracts of whole, dried sea hares. Purified, neat samples of isodactylyne deteriorated slowly at room temperature and hence no combustion analysis was obtained. Mass spectral analysis established that this new halogenated ether has the same elemental composition ($C_{15}H_{19}OBr_2Cl$) as dactylyne; indeed, the fragmentation patterns of **1** and **2** were virtually identical. The presence in **2** of a terminal acetylene group conjugated with a double bond was indicated by ir (3300, 2095 cm^{-1}) and uv data (λ_{max} 224 nm, shoulder at 233 nm; ϵ_{max} 15 500). The larger extinction coefficient in the uv spectrum of **2** relative to that of **1**[1] suggested the trans geometry for the double bond of the enyne group.[4]

Comparison of the NMR spectra of isodactylyne and dactylyne, see Figure 1, revealed great similarity in structure between the two, and at the same time confirmed that the enyne double bond of **2** is trans, as evidenced by the large coupling constant of the olefinic protons of the enyne group, δ 5.65 (broadened d, J = 16, 1–2 Hz), 6.24 (doubled t, J = 16, 7 Hz).[5] The triplet multiplicity in the δ 6.24 signal indicated coupling of this olefinic proton with an adjacent methylene group, thus confirming that the enyne side chain consisted of at least five carbons.

The olefinic proton signal centered at δ 5.80 (t, J = 8 Hz) and the entire upfield portion of the spectrum of isodactylyne resembled that of dactylyne to such a degree that a direct correspondence in structure at many points could be inferred. In fact, catalytic hydrogenation of **2** afforded a solid octahydromonodebromo product, mp 51.0–52.2 °C, M^+ *m/e* 342, 340, 338 ($C_{15}H_{28}BrClO$), which was identical with that obtained



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from dactylyne as judged by melting point, mixture melting point, R_f value, optical rotation, and ir, NMR, and mass spectra. This confirmed that **1** and **2** were identical with respect to carbon skeleton, size and location of the ether ring, type of halogen substitution on the ring, and relative stereochemistry of all ring substituents. In view of this confirmation of the C-5 length for both side chains in isodactylyne, the enyne chain could be formulated conclusively as shown in **2**.

The side chain bearing the vinyl bromide group is assigned

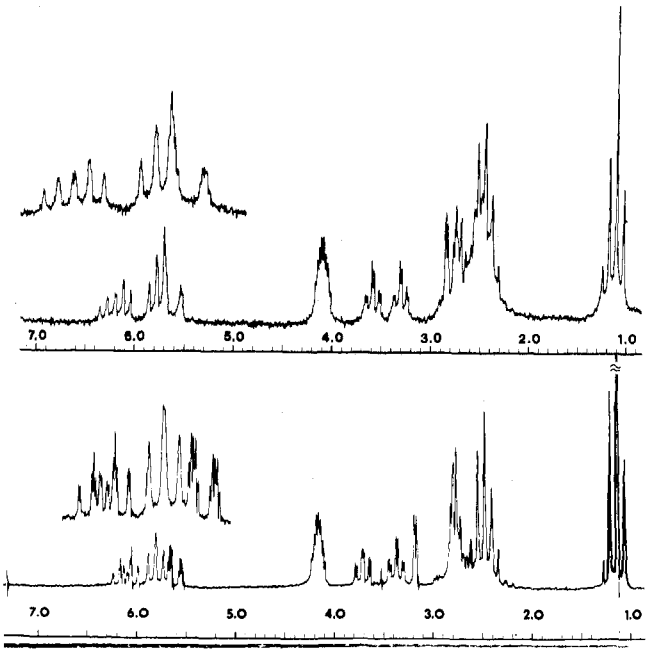

Figure 1. 100-MHz NMR spectra of dactylyne (**1**) (bottom) and isodactylyne (**2**) (top).

the same structure as the one in dactylyne on the basis of NMR data. The location of the bromine was established by showing that the signal due to the methylene unit of the ethyl group was a quartet (irradation at δ 1.13 collapsed the two intense lines centered at δ 2.50 to a singlet at that position). The vinyl bromide group is assigned the *E* configuration since the olefinic proton of this unit resonates at exactly the same position as does the corresponding proton in **1**, whereas a chemical shift difference would have been expected[6] if the configuration at this center were different for **1** and **2**.

Combination of the foregoing conclusions yields the structure **2** for isodactylyne, including the absolute stereochemistry implied thereby. An alternate structure for isodactylyne in which the location of the ring halogens is reversed and the configuration at all chiral centers is inverted would also give rise to the octahydromonodebromo derivative **3** upon hydrogenation. However, the configurtion shown for **2** is considered biogenetically more probable in the light of the established structure for dactylyne.

The assignment of the signals in the δ 3.2–4.2 region of the spectrum of isodactylyne parallels those for dactylyne.[2] Although we have isolated dactylyne and isodactylyne from a sea hare, these compounds undoubtedly come from the algae on which the mollusc feeds. Such a food chain source has been confirmed earlier for natural products isolated from *Aplysia californica*.[7]

Experimental Section[8]

Isolation of Dactylyne (1) and Isodactylyne (2). Specimens of *Aplysia dactylomela* collected in the environs of Bimini, Bahamas, were pierced and preserved whole in 2-propanol for shipment. Shortly after the specimens were received in our laboratories, sufficient water was added to bring the preservation solution to a 40/60 (v/v) water/alcohol mixture, and the specimens were allowed to soak for an additional 2 days. The bodies were then recovered by decantation and filtration, air dried (3.5 kg dry wt), broken into small pieces, and extracted in a Soxhlet apparatus with distilled hexane for 2–4 days. The hexane extract was filtered and the solvent evaporated to yield 204 g of a dark green oil.

A portion of this crude hexane extract (100 g) was chromatographed on Florisil (1500 g). One-liter fractions were collected employing the following elution scheme: hexane, fractions 1–7; benzene/hexane (1/3), fractions 8–11; benzene/hexane (1/1), fractions 12–18; benzene (2-l. fractions), fractions 19–22; ethyl acetate (one 8-l. fraction). Fractions

exhibiting similar TLC profiles were combined. A portion (2.5 g) of combined fractions 9–11 (16.45 g) was chromatographed on 40 g of thin layer mesh silica gel H using ether/hexane (5/95) as solvent and collecting 20-ml fractions. Dactylyne (175 mg) crystallized from the material obtained in fractions 18–20 (290 mg). Recrystallization from an ether/hexane mixture yielded large, colorless crystals roughly trapezoidal in shape which were utilized for x-ray crystallographic analysis.[2] After 29 fractions had been collected, one 250-ml fraction was collected and this yielded 170 mg of material, homogeneous by TLC. Chromatography of this fraction on thin-layer mesh silica gel gave 160 mg of isodactylyne (**2**). All attempts to crystallize isodactylyne were unsuccessful.

Isodactylyne had $[\alpha]^{24}D$ −8.06 ° (*c* 7.97, $CHCl_3$); R_f 0.46 (1:1 benzene/hexane, silica gel H); ir (neat) 3300, 3030, 2970, 2930, 2830, 2085 (weak), 1640, 1415, 1345, 1315, 1080 (br), 955, 870, 750, and 600 cm^{-1}; uv (isooctane) λ_{max} 224 nm (ϵ_{max} 15 500), with an inflection at 233 nm; 100-MHz NMR ($CDCl_3$), see Figure 1; mass spectrum (70 eV) *m/e* (rel intensity) M^+, 412 (3), 410 (4), 408 (2), 377 (1), 375 (2), 373 (1), 345 (2), 343 (3), 341 (2), 337 (7), 331 (16), 329 (10), 251 (3), 249 (5), 247 (1), 229 (3), 227 (4), 225 (1), 187 (5), 185 (10), 183 (10), 182 (8), 153 (15), 149 (24), 147 (28), 146 (15), 145 (14), 129 (11), 119 (29), 118 (18), 117 (51), 115 (14), 107 (11), 105 (20), 103 (31), 95 (11), 93 (10), 91 (34), 81 (23), 79 (30), 78 (18), 77 (19), 75 (10), 69 (13), 67 (65), 64 (100) base peak, 57 (15), 55 (18), 53 (27), 51 (12), 41 (40).

Pure dactylyne had mp 62.5–63.5 °C; $[\alpha]^{23}D$ −36.2° (*c* 15.2, $CHCl_3$); R_f 0.57 (1:1 benzene/hexane, silica gel H).

Octahydromonodebromodactylyne (3). A. From Dactylyne (1). To a stirred suspension of 30 ml of ethyl acetate containing a few milligrams of prereduced PtO_2 in a hydrogen atmosphere (1 atm) was added 105 mg of dactylyne dissolved in 10 ml of ethyl acetate. Hydrogenation was continued overnight, then the reaction mixture was filtered and the filtrate concentrated on a rotary evaporator to yield 77.8 mg (89.4%) of a clear, colorless oil which solidified after removal of the last traces of solvent under high vacuum. Recrystallization of the crude product from 95% ethanol yielded pure octahydromonodebromodactylyne (**3**), homogeneous by TLC: mp 51.4–52.5 °C; $[\alpha]D$ −0.90° (*c* 5.5 $CHCl_3$): ir ($CHCl_3$) 3000, 2960, 2860, 1450, 1415, 1370, 1345, 1310, and 1080 cm^{-1}; 60-MHz NMR[9] (CCl_4) δ 3.95 (m, 2 H, protons on the carbons bearing the halogens), 3.40, 320 (each 1 H, m, protons on carbons bearing oxygen), 2.67 (m, 2, methylene protons at C-4 of tetrahydropyran ring), 2.1–1.08 (m, 14 H), 0.95 (m, 6 H, terminal methyl group protons); mass spectrum (70 eV) *m/e* (rel intensity) 342 (2), 340 (6), 338 (5), 271 (14), 269 (53), 267 (37), 261 (4), 259 (10), 188 (16), 178 (13), 177 (20), 176 (15), 175 (59), 160 (10), 159 (9), 158 (24), 123 (97), 109 (13), 101 (37), 99 (19), 97 (33), 96 (42), 95 (16), 88 (22), 83 (93), 81 (88), 79 (12), 70 (40), 69 (55), 67 (84), 57 (25), 56 (32), 55 (100), 54 (22), 53 (25), 43 (76), 42 (20), 41 (94).

Anal. Calcd for $C_{15}H_{28}BrClO$: C, 52.87; H, 8.58; Br, 23.45; Cl, 10.40. Found: C, 53.47; H, 8.24; Br, 23.11; Cl, 10.09.

B. From Isodactylyne (2). Hydrogenation of 57.5 mg of **2** in the same manner as described above for **1** gave 36.7 mg (77.7%) of crude **3**. Recrystallization from 95% ethanol gave pure **3**, homogeneous by TLC: mp 51.0–52.5 °C; ir, NMR, and MS same as described in A above; mmp 51.5–53.3 °C; $[\alpha]D$ −0.90° (*c* 2.12, $CHCl_3$).

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Registry No.—**1**, 55306-12-2; **2**, 58001-90-4; **3**, 55229-33-9.

References and Notes

(1) Based on the Ph.D. Dissertation of D. J. Vanderah, University of Oklahoma, Norman, Okla., June 1975.
(2) F. J. McDonald, D. C. Campbell, D. J. Vanderah, F. J. Schmitz, D. M. Washecheck, J. E. Burks, and D. van der Helm, *J. Org. Chem.*, **40**, 665 (1975).
(3) See W. Fenical, *J. Phycol.*, **11**, 245 (1975), or articles cited in ref 2.
(4) R. M. Silverstein and G. C. Bassler, "Spectrometric Identification of Organic Compounds", Wiley, New York, N.Y., 1963, p 157; see also ref 5.
(5) Cf. spectra of laurencin [T. Irie, M. Suzuki, and T. Masamune, *Tetrahedron*, **24**, 4193 (1968)] and laureatin [T. Irie, M. Izawa, and E. Kurosawa, *ibid.*, **26**, 851 (1970)].
(6) L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", Pergamon Press, Oxford, 1969, p 184 ff.
(7) M. O. Stallard and D. J. Faulkner, *Comp. Biochem. Physiol. B*, **49**, 25 (1974).
(8) Melting points are uncorrected. Infrared spectra were taKen on a Beckman IR-8 spectrophotometer and ultraviolet spectra on a Cary Model 118 spectrophotometer using 1-cm matched quartz cells. NMR spectra were acquired on Varian T-60 or XL-100 spectrometers in the solvents specified; signals are reported in parts per million (δ) downfield from internal tetramethylsilane. Mass spectra were obtained on a Hitachi RMU-7 spectrometer and optical rotations on Perkin-Elmer 141 digital readout or Gaertner polarimeters. Microanalyses were obtained from Mr. E. Meier, Department of Chemistry, Stanford University, Palo Alto, Calif. Chromatographic adsorbents used were Florisil (Fischer, 100–200 mesh) and silicic acid (Mallinckrodt, silicAR CC-7 and Brinkmann TLC mesh).
(9) For NMR data at 100 MHz ($CDCl_3$) see ref 2.

Quassimarin, a New Antileukemic Quassinoid from *Quassia amara*[1,2]

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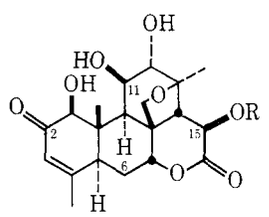
In the course of a continuing search for tumor inhibitors of plant origin, the sap of *Quassia amara* L.[3] (Simaroubaceae) was found to show significant activity in vivo against the P-388 lymphocytic leukemia in mice (PS) and in vitro against cells derived from human carcinoma of the nasopharynx (KB).[4] We report herein the fractionation of an active extract of *Q. amara* and the isolation and structural elucidation of a new antileukemic quassinoid,[5] quassimarin (**1**), and the companion quassinoid, simalikalactone D (**2**).



1, R = $COC(OAc)(CH_3)C_2H_5$
2, R = $COCH(CH_3)C_2H_5$

Fractionation of the dried sap, guided by assay against the KB and PS systems, revealed that the inhibitory activity was concentrated, successively, in the ethyl acetate layer of an ethyl acetate–water partition, the aqueous methanol layer of a 10% aqueous methanol–petroleum ether partition, and in the aqueous methanol layer of a 20% aqueous methanol–carbon tetrachloride partition. Column chromatography of the final aqueous methanol soluble material on SilicAR CC-7 yielded KB- and PS-active fraction D upon elution with 2% methanol in chloroform. Rechromatography of fraction D, first on silica gel 60 with isopropyl alcohol in dichloromethane as eluent, then on SilicAR CC-7 with acetone in hexane as eluent, gave quassimarin (**1**) and simalikalactone D (**2**).[6]

Elemental analysis and high-resolution mass spectrometry established a molecular formula of $C_{27}H_{36}O_{11}$ for quassimarin (**1**). The presence of an α-acetoxy-α-methylbutyrate ester was indicated by peaks in the mass spectrum at *m/e* 143 $[CO\text{-}C(OAc)(CH_3)C_2H_5]\cdot^+$, 115 $[C(OAc)(CH_3)C_2H_5]\cdot^+$, and 83 $[COC(CH_3)=CHCH_3]\cdot^+$, and by a dominant high mass fragment ion at *m/e* 358 corresponding to $M^+ - H_2O -$ HOOC-$C(OAc)(CH_3)C_2H_5$. Furthermore, the NMR spectrum contained signals for primary, tertiary, and acetate methyl groups assignable to the ester. Lithium aluminum hydride reduction of **1** afforded 2-methyl-1,2-butanediol. A one-proton doublet at τ 3.52 (J = 14 Hz) in the NMR spectrum of **1** confirmed the point of ester attachment to be at C-15.[7]

Irradiation of the C-15 proton doublet at τ 3.52 led to the