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ENT-13-EPICONCINNDIOL FROM THE RED ALGA *CHONDRIA TENUISSIMA* AND ITS ABSOLUTE CONFIGURATION

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Key Word Index—*Chondria tenuissima*; Rhodomelaceae; marine natural products; brominated diterpene; *ent*-13-epiconcinndiol; X-ray crystallography.

Abstract—The bromo-diterpene, *ent*-13-epiconcinndiol, has been isolated from the marine red alga *Chondria tenuissima*. Its absolute configuration was determined by X-ray crystallography.

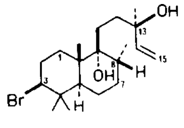
INTRODUCTION

The large number of halogenated metabolites isolated from the marine algae comprise a small group of labdane-type bromo-diterpenes: concinndiol [1, 2], aplysin-20 [3], isoconcinndiol [4, 5] and pinnatols [6], all isolated from *Laurencia* species (Rhodomelaceae). On continuation of our phytochemical work on marine algae collected from Turkish waters, investigations of *Chondria tenuissima*, an alga of the same family, resulted in the isolation of a new compound which proved to be an isomer of a bromo-diterpene formerly isolated from *L. concinna*.

RESULTS AND DISCUSSION

Compound **1** was isolated from the chloroform soluble part of the chloroform–methanol (1:1) extract by a combination of silica gel column and prep. TLC. The compound, with a mp 101.5–102.5° and optical rotation of $[\alpha]_D^{25} = +17.34°$ ($CHCl_3$; c 0.445), had a molecular formula of $C_{20}H_{35}BrO_2$ as established by HRMS. The IR spectrum contained characteristic bands for hydroxyl (3350 cm^{-1}), vinyl (3083, 986, 910 cm^{-1}) and *gem*-dimethyl (1383, 1365 cm^{-1}) functions. The ^1H NMR spectrum (360 MHz, $CDCl_3$) showed four singlets at δ1.29, 1.06, 0.95 and 0.92 due to four tertiary methyl groups and a three proton doublet at δ0.85 (*d*, $J = 6.5$ Hz) derived from one secondary methyl group. A double doublet centred at δ4.04 (1H, *dd*, $J = 11.8$ and 5.0 Hz) indicated a secondary bromo group with an equatorial bromo substituent attached to a cyclohexane ring, while signals at δ5.82 (1H, *dd*, $J = 17.5$ and 10.8), 5.20 (1H, *dd*, $J = 17.5$ and 1.7) and 5.10 (1H, *dd*, $J = 10.8$ and 1.7) revealed a terminal vinyl group. As compound **1** failed to form an acetylation product, the two protons which are exchangeable with D_2O have to be assigned to the tertiary hydroxyl groups. The ^{13}C spectrum (see Experimental) was very similar to that reported for concinndiol [4].

The complete structure and stereochemistry of compound **1** obtained by X-ray crystallography revealed the isolated compound to be 13-epiconcinndiol (Fig. 1). Direct comparison of the ^1H and ^{13}C NMR spectra of **1** and those of authentic (±)-13-epiconcinndiol showed the same features for both compounds. Yamaguchi *et al.* [7] have synthesized rac-13-epiconcinndiol and assigned the stereochemistry to that of concinndiol (**2**), following the original work of Fenical *et al.* [1] who determined the structure and absolute configuration of concinndiol by X-ray methods. The absolute configuration of **1** was proved unequivocally to be the enantiomer of 13-epiconcinndiol in three different ways: the Hamilton *R*-factor test [8], Rogers' η refinement [9], and measurements of 20 selec-



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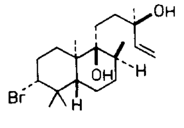

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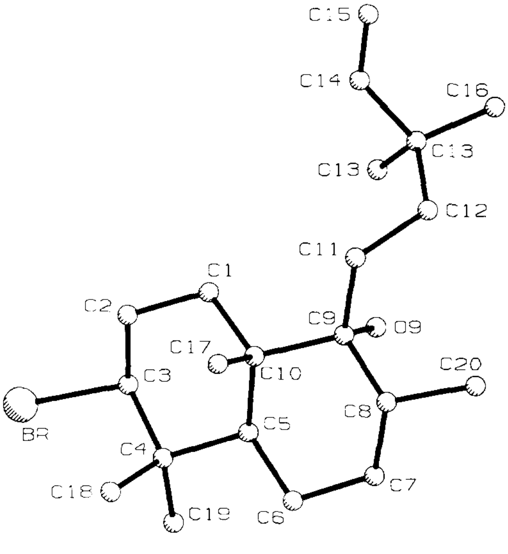

Fig. 1. Perspective view of *ent*-13-epiconcinndiol.

ted Bijvoet pairs [10]. According to these results the diterpene **1** differs in its absolute configuration from that of the other naturally occurring bromo-diterpenes.

From the polar fraction of the extract, 2-*O*-α-D-galactopyranosylglycerol (fluoridoside) and, upon acetylation, its hexa-acetate were isolated. This galactoside has been obtained previously from the algae *Irideae laminarioides* [11] and *Laurencia pinnatifida* [12].

EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR spectra with TMS as int. ref.

Isolation. Chondria tenuissima was collected during Sept. 1987 at Sarköy from the Marmara Sea, Turkey. A voucher specimen was deposited in the Herbarium, Department of Systematic Botany, Faculty of Science, University of Ege, Izmir, Turkey. The air-dried alga (1.4 kg) was extracted with $CHCl_3$–MeOH (1 : 1) to yield 10.8 g crude extract after solvent evapn *in vacuo*. The salts, which were deposited from the soln during concentration, were filtered off. The $CHCl_3$ soluble part (extract A, 7.2 g) and the remaining polar fraction (extract B, 3.6 g) were worked up separately. Extract A was chromatographed on a silica gel column eluted successively with petrol, petrol–C_6H_6, C_6H_6, C_6H_6–$CHCl_3$, $CHCl_3$ and $CHCl_3$–MeOH mixtures. Appropriate fractions eluted with $CHCl_3$ were further separated by prep. TLC ($CHCl_3$–MeOH, 30 : 1) to give **1** (120 mg). A portion of the extract B, after purification by prep. TLC ($CHCl_3$–MeOH, 1 : 1) afforded the major constituent fluoridoside in 0.075% total yield. ^{1}H NMR (80 MHz, D_2O): 5.16 (1H, *d*, $J = 2.8$ Hz). The remaining extract B was treated with Ac_2O–pyridine at room temp. for 2 days to give the hexa-acetate of fluoridoside. Purification on silica gel followed by crystallization from *n*-hexane–$CHCl_3$ yielded colourless needles. Its mp, [α] and ^{1}H NMR data were identical with those reported previously [11].

ent-13-*Epiconcinndiol* (**1**). Colourless crystals, mp 101.5–102.5° (from *n*-hexane–$CHCl_3$); $[\alpha]_D^{25} = +17.34°$ ($CHCl_3$; *c* 0.445) $C_{20}H_{35}BrO_2$ [Found: 368.1716; calc. for $^{79}M - H_2O$, $C_{20}H_{33}{}^{79}BrO$, 368.1715] IR ν_{max}^{KBr} cm^{-1}: 3350, 3083, 2970, 2940, 1728, 1640, 1462, 1412, 1383, 1365, 1320, 1278, 1137, 1110, 986, 940, 910. ^{1}H NMR: (see text). ^{13}C NMR (20.15 MHz, $CDCl_3$): δ15.9, 16.6, 18.4, 23.3, 28.3, 29.3, 30.9, 31.1, 31.5, 33.8, 36.8, 37.1, 39.7 (C-4), 43.7 (C-10), 47.3 (C-5), 70.0 (C-3), 73.7 (C-13), 76.3 (C-9), 112.2 (C-15), 144.6 (C-14). EIMS 70 eV, *m/z* (rel. int.): 388, 386 $[M]^+$ (each *ca* 0.3), 370, 368 (6.5 : 6.5), $[M - H_2O]^+$, 289 (4) $[M - H_2O - Br]^+$, 271 (3) $[M - 2H_2O - Br]^+$, 189 (8), 151 (52), 107 (47), 95 (48), 93 (46), 91 (51), 81 (100). CIMS (NH_3) 120 eV, *m/z* (rel. int.): 388, 386 (20 : 18), 371, 369 and 370, 368 (63 : 66 and 63 : 62 resp.) $[M + 1 - H_2O]^+$ and $[M - H_2O]^+$, 353, 351 (79 : 92) $[M + 1 - 2H_2O]^+$, 289 (48), 271 (100), 191 (20), 151 (73), 109 (39), 95 (38), 81 (48).

X-Ray structure analysis of compound **1**. Suitable crystals were obtained from *n*-hexane–$CHCl_3$ (20 : 1) as colourless transparent needles. Crystal data: orthorhombic space group $P2_12_12_1$ $a = 7.795(2)$, $b = 9.282(5)$, $c = 28.095(9)$ Å, $z = 4$, $D = 1.27$ g/cm^3, $D_{meas} = 1.26$ g/cm^3. Sample dimensions: 0.3 × 0.2 × 0.8 mm. A total of 1617 unique reflections were measured using Ni-filtered CuK_α radiation in Ω-scan mode on a Nicolet R3m diffractometer. 1303 reflections were considered as observed with $I > 3\sigma(I)$ up to $2\theta_{max} = 114°$. Absorption correction was applied ($\mu = 28.1$ cm^{-1}). The structure was solved by direct methods using SHELXTL [13] and subsequent difference Fourier maps. The hydrogen atoms were calculated from the positions of the carbons to which they are bound. Final refinement cycles reached an *R*-factor of 6.2% (hydrogens included).

Determination of the absolute configuration. The absolute configuration was determined with three different methods. (i) Hamilton *R* factor test: Both enantiomers were refined (hydrogens not included) to convergence: *ent*-13-epiconcinndiol 9.03%, 13-epiconcinndiol 9.32%. This result points clearly to the first enantiomer. The refinement was carried out for 1353 reflections and 208 parameters; The *R* factor ratio 1.032 is significant at a level of less than 0.005. (ii) Rogers' η refinement for the *ent*-13-epiconcinndiol yielded $\eta = 0.9$ with a $\sigma = 0.15$ and indicated again the correctness of our absolute structure assignment. (iii) At least Bijvoets method was carried out. Twenty reflections with a relatively significant anomalous-dispersion portion were selected by aid of an option provided in the SHELXTL programs (HKLF-5). The intensities of these reflections and their Friedel opposites were measured several times in an alternating procedure. All intensities were corrected for absorption. The comparison of the calculated and measured Bijvoet differences of the 20 reflections showed without exception the predicted sign. That is the strongest check for the correct choice of the absolute configuration. Full crystal data are deposited at the Cambridge Crystallographic Data Centre.

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