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SESQUITERPENES AND GERANYLGERANYLGLYCEROL FROM THE BROWN ALGAE *TAONIA LACHEANA* AND *TAONIA ATOMARIA* F. *CILIATA*: THEIR CHEMOTAXONOMIC SIGNIFICANCE

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Key Word Index—*Taonia lacheana*; *Taonia atomaria* f. *ciliata*; Dictyotaceae; sesquiterpenes; aromadendranes; geranylgeranylglycerol.

Abstract—*Taonia lacheana* is a very recent addition to the small *Taonia* genus. The chemical composition of the new species is characterized by the presence of aromadendrane sesquiterpenes [(+)-spathulenol, (+)-11-epispathulenol and (−)-7,11-dihydroxyaromadendrane] and (−)-(*R*)-1-geranylgeranylglycerol. A related species from which the last compound had been previously isolated, misidentified as *Dilophus fasciola* and now recognized to be *T. atomaria* f. *ciliata*, has been re-investigated confirming the absence of aromadendrane derivatives. The two Mediterranean *Taonia* species differ sharply from the Canarian *T. atomaria*, that is known to elaborate merediterpenoids.

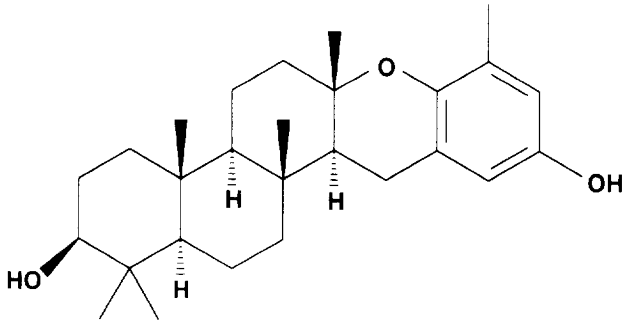
INTRODUCTION

The small algal genus *Taonia* includes *T. lennebackerae* from the Pacific coasts of North America, *T. australasica* found in the south-eastern Australian waters, and *T. atomaria* widely distributed in the Atlantic Ocean and in the Mediterranean Sea. A variety of this last species, *T. atomaria* f. *ciliata* is common in the Mediterranean and occasionally also found in the Atlantic. Chemical examination of *T. atomaria* from the Canary Islands has resulted in the isolation of several meroditerpenoids [1–5], exemplified by taondiol (**1**) [1], while from *T. australasica* the diterpene taonianone [6] has been isolated. A recent report on the chemistry of *T. atomaria* from the northern Adriatic Sea [7] describes the identification of the sesquiterpenes 4-cadinene, cadinane-4(14),5-diene, (−)-germacrene D, axenol (−)-cubebol (**2**) and epi-cubebol, but does not mention any of the meroditerpenoids from the Canarian species.

The recent report from local botanists of a new *Taonia* species, *T. lacheana* [8], collected at *ca* − 12 m near Lachea Island (Gulf of Catania) prompted us to study its chemical composition, and in the present paper we report the results obtained. Moreover, we have re-examined a brown alga previously investigated under the name *Dilophus fasciola* [9–12], whose classification has been revised into *T. atomaria* f. *ciliata*.

RESULTS AND DISCUSSION

Chromatographic fractionation of the ethylacetate extract from air-dried and ground thalli of *T. lacheana* gave, along with ubiquitous constituents such as chlorophylls, fats and sterols, two main fractions (A and B), of medium and high polarity, respectively. The main component of fraction A, homogeneous in TLC in different solvent systems, when subjected to ^{13}C NMR spectroscopy and GC-mass spectrometry turned out to be a mixture of two isomeric compounds, which were separated by flash-chromatography on $AgNO_3$-impregnated silica gel. The major component, **3**, on the basis of its two-dimensional NMR data and $[\alpha]_D$ value, was identified as the sesquiterpene 11β-hydroxy-1β,8α-aromadendrene [(+)-spathulenol] [13–17]. The isomeric **4** was also a dextrorotatory compound, having ^1H and ^{13}C NMR spectra very similar to those of **3** (see Experimental), indicative of the presence of the same carbon skeleton. Moreover, the close similarity of the coupling constants for the cyclopropane protons H-2 and H-4 with the corresponding ones in the spectrum of **3** suggested the identity of the stereochemistry at the bridgehead carbons C-2 and C-4. Thus, the structural differences between **3** and **4** were restricted to the stereochemistry of the chiral centres at C-1, C-4 and C-8. The possibility that **4** could be the known isomer 11β-hydroxy-1β, 8β-aromadendrene (allospathulenol) was ruled out on the basis of the differences between the NMR data for **4** and those reported for allospathulenol [18]. Assuming that the stereochemistry of the junction of the five- and seven-membered cycles is identical to that of spathulenol and taking into account the ^{13}C NMR shift of the carbinyl caron (δ74.7), with respect to the corresponding value for spathulenol (δ81.0) [17], we suggest for **4** the



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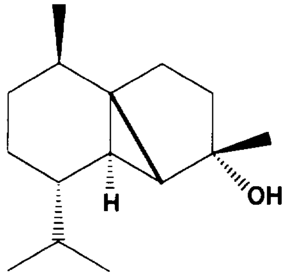

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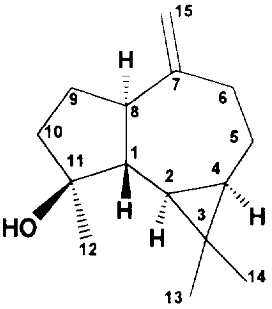

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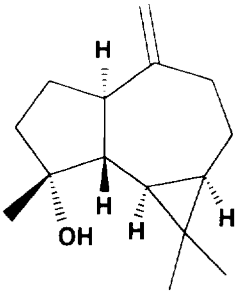

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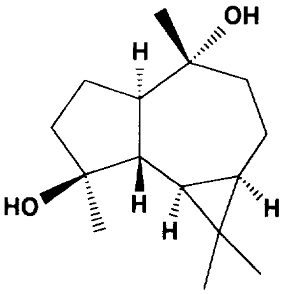

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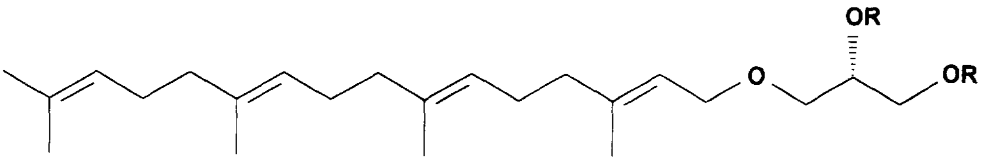
structure of (+)-11α-hydroxy-1β,8α-aromadendrene [(+)-11-epispathulenol]. To the best of our knowledge, this is the first reported natural occurrence of this sesquiterpenoid, whose enantiomer has been obtained by synthesis [15].

Flash-chromatography of the more polar fraction (B) from the extract of *T. lacheana* gave the main component (**5**) in the pure state. A second subfraction was subjected to acetylation followed by chromatographic fractionation to yield a minor component, **4**, and the diacetate **7**. Compound **5** was easily identified by spectral analysis as the known (−)-(*R*)-1-*O*-geranylgeranylglycerol [9, 19]. The minor component of fraction B, not acetylated under the conditions adopted, showed ^{1}H and ^{13}C NMR resonances strongly resembling those of **3** and **4**, the main difference being the absence of the low-field exomethylene signals, which were replaced by the resonances for a second tertiary alcoholic group (^{1}H: δ1.16, Me, *s*; ^{13}C: δ74.8, *s*; 20.0, *q*). A literature search allowed **6** to be identified as (−)-7α,11β-dihydroxy-1β,8α-aromadendrane on the basis of IR, NMR and mass spectral data and $[\alpha]_D$ measurement [18, 20–21].

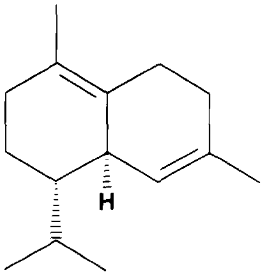
Metabolites **3**, **4** and **6** are the first examples of aromadendrane sesquiterpenes isolated from brown algae. Compounds of this class, in particular (+)-spathulenol, occur in many plant genera, while aromadendrane diols are less frequently encountered [18]. In this connection, mention should be made that the (+)-enantiomer of **6** has been isolated from the soft coral *Sinularia mayi* [20].

Identification of **5** in the extract of *T. lacheana* prompted us to reconsider the only other reported occurrence of this compound in a brown alga, *D. fasciola* [9]. After a botanical re-examination of this seaweed, which led to modification of its classification into *T. atomaria* f. *ciliata*, a careful analysis of the ethyl acetate extract was carried out to determine the possible occurrence of aromadendrane sesquiterpenoids. In addition to metabolites identified previously [δ-cadinene (**8**), 4,10-epoxymuurolane (**9**), 1,4-epoxycadinane (**10**), cubenol (**11**) and geranylgeranylglycerol (**5**)] [9, 10, 12], the sesquiterpene **2** [22, 23], identified in an extract of northern Adriatic *T. atomaria* [15], has been isolated, while no evidence was obtained for the presence of aromadendrane derivatives.

The chemical composition of *T. lacheana*, characterized by aromadendrane sesquiterpenes, differs sharply from that of *T. atomaria* f. *ciliata*, that elaborates sesquiterpenes mostly with the cadinane [10, 12] or germacrane [11] skeleton. However, it is noteworthy that the two species, which occur in the same waters, share the ability to synthesize **5**, a metabolite not found until now



5 R = H
7 R = Ac



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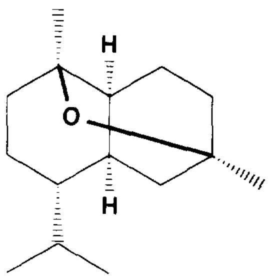

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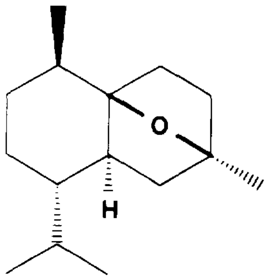

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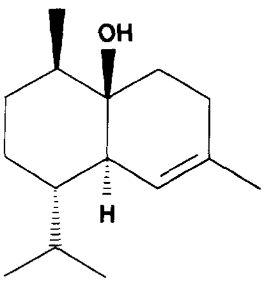

11

in any other alga. The new species also differs chemically from the northern Adriatic *T. atomaria*, again characterized by the presence of sesquiterpenes mostly with cadinane or germacrane skeletons [15]. The chemical composition of the Canarian *T. atomaria* is completely different; several merediterpenoids have been isolated which are not found in other species belonging to the same genus. The fact that metabolites of this class, for instance taondiol and atomaric acid, have been isolated also from species of the genus *Stypopodium* [24–26], not easily separated morphologically from *Taonia*, suggests the possibility that a botanical misidentification is at the origin of the apparently anomalous chemical composition of the Canarian *T. atomaria*.

EXPERIMENTAL

General. Mps were determined on a Kofler hot stage apparatus. Optical rotations were taken at 25° on a Perkin-Elmer 141 polarimeter in $CHCl_3$ soln. EIMS spectra were recorded on a Kratos MS 50 instrument, at 70 eV. IR spectra were run on a Perkin-Elmer 684 spectrophotometer in $CHCl_3$ solution. ^1H NMR spectra were recorded at 200 MHz on an AC-200 Bruker instrument. ^{13}C NMR spectra were run at 50.13 MHz on an AC-200 Bruker instrument equipped with $^1H/^{13}C$ dual probe. All NMR experiments were performed in $CDCl_3$ with TMS as int. standard. GC-MS analyses were carried out on a Hewlett-Packard gas chromatograph, model 5890, equipped with a MS computerized system, model 5971A, ionization voltage 70 eV, electron multiplier 1700 V, ion source temp. 180°. GC conditions: HP-1 dimethylpolysiloxane capillary column (25 m × 0.2 mm). The oven temp. was held at 60° for 6 min, then programmed from 100° to 250° at 3° min^{-1}.

Plant material. Samples of the algae were collected at a depth of, respectively, *ca* – 3 m (*T. atomaria* f. *ciliata*) *or ca* – 12 m (*T. lacheana*) near Lachea Island in April, 1993. Herbarium specimens are deposited at the Dipartimento di Botanica, Università di Catania. Carefully selected and classified thalli of both species were air-dried in the shadow and ground.

Extraction and fractionation. The powdered material of *T. lacheana* (100 g) was extracted with EtOAc (1.5 l × 3) at room temp. under continuous stirring. Evapn of the solvent under red. pres. afforded a dark-green oil (2.3 g), which was gross-fractionated by open column chromatography (Lichroprep Si-60 Merck, 40–63 μm, increasing amounts of Et_2O in *n*-hexane as the eluent) to give two main frs (A, B). The less polar frs (A, 180 mg) was subjected to flash-chromatography (Lichroprep Si-60, 25–40 μm, eluent i-Pr_2O-hexane from 10 to 60%). The major subfr., homogeneous in TLC in various solvent systems was revealed to be a mixture of two isomeric compounds, **3** and **4** (^{13}C NMR, GC-MS), that were separated by PLC on $AgNO_3$-impregnated silica gel (Lichroprep Si-60, 25–40 μm; *i*-Pr_2O–*n*-hexane, 2 : 3, as the eluent). Compound **3** (29 mg) was identified as (+)-spathulenol basing on 2D NMR analysis (COSY, one bond HETCOR, long-range HETCOR) and $[\alpha]_D$ value { + 7.2° (*c* 1.3 in $CHCl_3$); lit. + 5.7° [14] and − 7.5° for (−)-spathulenol [15]}. Compound **4** (9 mg) was identified as (+)-11-epispathulenol, mp 102–103°; MS: *m/z* 220 $[M]^+$, 205, 202, 169, 187, 160, 159 (base) 133; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 3075, 2920, 1645, 1455, 1375, 1100, 885; $[\alpha]_D$ = + 2.5° (*c* 0.5 in $CHCl_3$); $[\alpha]_D$ = + 2.0 (*c* 0.7 in Me_2CO) {lit. − 5° in Me_2CO [15]}. ^{13}C NMR δ157.5 (*s*, C-7); 103.2 (*t*, C-15); 74.7 (*s*, C-11); 56.4 (*d*, C-8 and C-1); 42.3 (*t*, C-10); 38.8 (*t*, C-6); 31.4 (*d*, C-2); 29.7 (*d*, C-4); 29.1 (*q*, C-12); 28.4 (*t*, C-9); 28.2 (*q*, C-13); 25.9 (*t*, C-5); 19.2 (*s*, C-3); 16.1 (*q*, C-14); ^{1}H NMR: δ4.74 (1H, *bs*, H-15a); 4.66 (1H, *bs*, H-15b); 2.46 (1H, *m*, H-6a); 2.22 (1H, *m*, H-8), 2.0–1.0 (7H, overlapped *m*); 1.23 (3H, *s*, 12 Me) 1.09 (3H, *s*, 14 Me); 1.01 (3H, *s*, 13Me); 0.99 (1H, *m*, H-5a); 0.62 (1H, *ddd*, J = 10.5, 9.5, 7 Hz, H-4); 0.32 (1H, *t*, J = 9.5 Hz, H-2). The more polar fr. (B, 150 mg) was further chromatographed (flash) on Lichroprep Si-60 (25–40 μm) eluting with a gradient from 50 to 80% of Et_2O in *n*-hexane and afforded 16 mg of pure (−)-(*R*)-1-*O*-geranylgeranylglycerol (**5**), identity based on MS, ^{1}H and ^{13}C NMR spectra [9, 19] and $[\alpha]_D$ value { − 2.2° (*c* 1.3 in $CHCl_3$); lit. − 2.1° [9], − 2.3° [19]}. A second subfr. was exposed overnight to vapours of Ac_2O and pyridine at room temp. and then subjected to flash-chromatography (Lichroprep Si-60 25–40 μm, increasing concn of Et_2O in *n*-hexane as the eluent) to afford 6 mg of (−)-7α,11β-dihydroxy-1α,8β-aromadendrane (**6**), identified via MS, ^{1}H and ^{13}C NMR data [20, 21] and $[\alpha]_D$ value { − 17.5° (*c* 0.2 in $CHCl_3$; lit. − 17° [21], − 22.9° [20]}, and 32 mg of (−)-2(*R*),3-diacetoxy-1-(*R*)-*O*-geranylgeranylglycerol (**7**) [9], MS: *m/z* 448 $[M]^+$; ^{13}C NMR: δ170.5, 170.2 (*s*, $MeCO_2$); 140.8 (*s*, $MeC{=}CHCH_2O$); 135.3, 134.8, 131.1 (*s*, $MeC{=}$); 124.3, 124.1, 123.7, 120.3 (*d*, $=CH$); 70.3 (*t*, CH_2O); 67.8 (*d*, *C*HOAc); 67.7, 62.9 (*t*, CH_2O); 39.6 (*t*, $3 \times CH_2C{=}$); 26.7, 26.5, 26.3 (*t*, $CH_2CH{=}$); 25.6 (*q*, *Me*CMe); 20.9, 20.6, 17.6, 16.4 (*q*, 2 × *Me*COO and 4 × *Me*C=); ^{1}H NMR: δ5.30 (1H, *t*, J = 7.0 Hz, $=CHCH_2O$); 5.08 (3H, *bt*, $=CH$); 5.18 (1H, *m*, C*H*OAc); 4.40–4.10 (4H, *m*, $2 \times CH_2O$); 3.97 (2H, *d*, J = 7.0 Hz, $=CHCH_2O$); 2.06 (3H, *s*, MeCOO); 2.03 (3H, *s*, MeCOO); 1.98 (12 H, *m*, $3 \times {=}CHCH_2CH_2$); 1.65 (6H, *bs*, 2 × MeC=); 1.57 (9H, *s*, 3 × MeC=).

Extraction and fractionation. The powdered material of *T. atomaria* f. *ciliata* (180 g) was extracted with EtOAc (2 l × 3) and the extract (dark-green oil, 11.7 g) was fractionated by open-column chromatography (Lichroprep Si-60 40–63 μm, increasing amounts of Et_2O in *n*-hexane as the eluent) to afford six main fractions, A–F, in order of increasing polarity. Fr. A (2.6 g) was subjected to GC-MS analysis and the known sesquiterpene δ-cadinene (**8**) was identified on the basis of GC retention time, computer matching with NBS library and comparison of fragmentation pattern with that reported in lit. [27]. Fr. B (630 mg) was subjected to flash-chromatography on Lichroprep Si-60 (25–40 μm), with EtOAc–*n*-hexane (0.5 : 99.5) as the eluent. The following compounds were isolated in order of increasing polarity: 4,10-epoxymuurolane (**9**) (160 mg), 1,4-epoxycadinane (**10**) (110 mg) and cubenol (**11**) (60 mg), all of them identified on the basis of reported spectral data (MS, IR, NMR) [10, 12, 28, 29]. The third fr. (C, 1.5 g), was analysed by GC-MS and three of the major constituents were identified as the known fatty acids myristic, palmitoleic and linolenic. Fr. D (1.3 g) was flash-chromatographed (Lichroprep Si-60, 25–40 μm, gradient from 0 to 5% MeOH in CH_2Cl_2). A subfr. of intermediate polarity was subjected to further flash-chromatography (Lichroprep DIOL, 25–40 μm, gradient from 0 to 12% of EtOAc in *n*-hexane) to afford (−)-cubebol (**2**) (45 mg), identified via MS, IR, ^{1}H and ^{13}C NMR spectra [7, 23] and $[\alpha]_D$ value { − 46.1° (*c* 1.2 in $CHCl_3$); lit. − 48.3° [23] − 67° [7]}. Fr. E (4.3 g) was not subjected to further analysis since preliminary TLC showed that chlorophylls and sterols were the main constituents. The more polar fr. (F, 1.2 g) was subjected to flash-chromatography (Lichroprep Si-60, 25–40 μm, elution with a gradient of Et_2O in *n*-hexane from 50 to 80%) and gave geranylgeranylglycerol (55 mg), identical in all respects to compound **5** isolated from *T. lacheana*.

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