Structure of a Pro-1,4-dimethylazulene Guaianolide from *Thapsia garganica* L.

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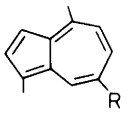
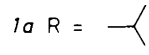
From an extract of the fruit of *Thapsia garganica* L. *(Apiaceae)* a novel guaianolide was isolated and structure elucidated as a slovanolide esterified with acetic, butanoic, and 2-methylbutanoic acid. Pyrolysis converted the slovanolide into 1,4-dimethylazulene suggesting it as a precursor for the 1,4-dimethylazulene found in the essential oil prepared by steam destillation of the fruit.

Guaiazulene (*1a*) and linderazulene (*2*) are the principal blue and purple pigments in deep sea gorgonians.[1,2] In contrast, the azulenes found in a number of essential oils of terrestrial sources are thought not to be genuine, but formed during steam destillation involved in the preparation of these oils. Thus artabsin (*3*), matricin (*4a*), and epimatricin (*4b*) isolated from extracts of *Artemisia* species produce chamazulene (*1b*) under the mild conditions of steam destillation.[3,4]



1a R =



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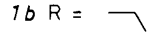
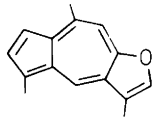


1c R = -H



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Elimination of water and the carboxylic acids esterified with the alcoholic functions of the guaiane skeleton followed by decarboxylation of the intermediately formed dihydrochamazulene-11-carboxylic acid and air oxidation yield *1b*.[4] The trinorsesquiterpenoid azulene, 1,4-dimethylazulene (*1c*) has been found in the oils of *Ruta graveolens*,[5] *Pimpinella nigra*[6] and *Eriocephalus punctualis*,[7] but this paper is the first report on a pro-1,4-dimethylazulene.

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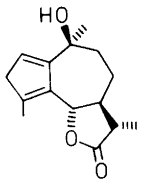
Results and discussion

Steam destillation of the fruits of *Thapsia garganica* for 6 h yielded an intensively blue oil. The major blue principle was isolated by dry column chromatography and, by ^{1}H NMR and electron impact mass spectrometry, shown to be 1,4-dimethylazulene (*1c*).

Thin layer chromatography of an ethanolic extract of the fruits showed that one of the components was visualized as an intensively blue spot simply by heating the plate to 110 °C for 10 min. After extracting the blue area with ether, the dye was shown to be identical with *1c* as evidenced by GC–MS. The precursor of *1c* was isolated in pure state by column chromatography and semi-preparative HPLC. Inspection of the ^{1}H NMR spectrum revealed the presence of an acetyl, a butanoyl and a 2-methylbutanoyl moiety. The presence of these acyl moieties was further em-



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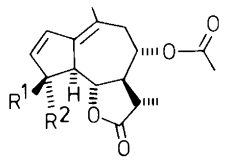
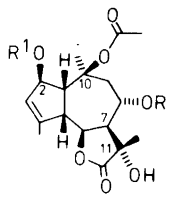
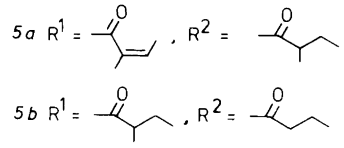
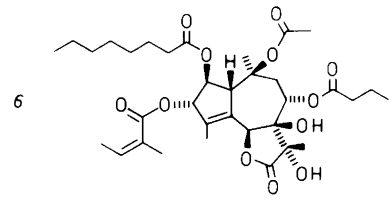


4a R^1 = -CH_3, R^2 = -OH

4b R^1 = -OH, R^2 = -CH_3







phasized by the dominating fragmentation pattern in the CI–MS spectrum, in which fragments corresponding to the protonated molecular ion minus acetic acid, the protonated molecular ion minus acetic and methylbutanoic acid, the protonated molecular ion minus acetic, methylbutanoic, and butanoic acid, and the protonated molecular ion minus acetic, methylbutanoic, butanoic and pyruvic acid were observed. The last named ion had the same *m/z* value as protonated *1c*. This fragmentation pattern is analogous to that observed for some polyoxygenated guaianolides.[8] An examination of the two-dimensional homonuclear $^1H-^1H$ chemical shift correlation diagram (COSY) enabled elucidation of the proton sequence depicted in formula *5*. Thus an allylic coupling between Me15 and H3, a vicinal coupling between H3 and H2, H2 and H1, H1 and H5, H5 and H6, H6 and H7, H7 and H8, and vicinal couplings between H8 and the two protons attached to C9 were observed. Examinations of the ^1H and ^{13}C NMR spectra revealed that, besides the mentioned moieties, the molecule contained only two sp^3 hybridized quaternary carbons each bound to a methyl group and oxygen (C10 and C11), and a carbonyl group. The molecular weight calculated on basis of these functionalities was in agreement with the CI–MS spectrum. The ease by which the compound was transformed into an azulene implied that the compound was a hydroazulene derivative, proving that C4 must be bound to C5, and C1 to C9 via one of the unlocalized quaternary carbons. The low chemical shift value of H6 compared to those of H2 and H8 indicated that this proton was attached to a γ-lactone ring. The high δ values of H2 and H8 proved O2 and O8 to be acylated; the

Table 1. ^1H NMR data of compound *5a* and *5b*. Chemical shifts (δ values) of $CDCl_3$ solutions. Multiplicities and *J* values (Hz) are shown in parentheses.

Proton	Compound *5b*	Compound *5a* (From Ref. 11)
1	3.27 (dd; 7.8; 3.4)	3.29 (dd; 7.5; 3.9)
2	5.72 (m)	5.80 (m)
3	5.60 (q; 1)	5.66 (m)
5	3.08 (m)	3.07 (m)
6	4.62 (dd; 11.7; 9.3)	4.64 (dd; 11.8; 9.3)
7	3.01 (dd; 10.2; 9.3)	3.04 (dd; 10.5; 9.3)
8	5.74 (ddd; 11.2; 10.2; 2.2)	5.70 (ddd; 11.1; 10.5; 2.2)
9	2.52 (dd; 15.3; 2.2)	2.59 (dd; 15.5; 2.2)
9′	2.10 (dd; 15.3; 11.2)	2.12 (dd; 15.4; 11.1)
13	1.59 (s)	1.58 (s)
14	1.39 (s)	1.43 (s)
15	1.92 (d; 1)	1.92 (b)
OH	2.41 (br)	

The signals due to the acyl moieties appear at the following δ values: *5b:* acetyl, 2.04 (s); α-metylbutanoyl, 2.32 (m), 1.11 (d; 7.0), 1.65 (m), 1.44 (m), 0.88 (t; 7.5); butanoyl, 2.29 (t; 7.5), 1.65 (m), 0.94 (t). *5a:* acetyl, 2.05 (s); α-metylbutanoyl 2.36 (m), 1.18 (d), 1.73 (m), 1.45 (m), 0.91 (t); angeloyl, 1.86 (br); 6.05 (qq); 1.95 (dq).

elimination of pyruvic acid upon CI–MS showed that O11 must be part of a hydroxy group implying that the third acyl group was attached to O10.

Shortage of isolated compound only allowed studies of the stereochemistry by spectroscopic means. An unequivocal proof for the stereochemistry would be possible only if all 256 stereoisomers of 2,8,10-triacyloxy-11-hydroxyguaia-3-en-6,12-olide were described. Unfortunately, this is not the case. However, especially the ^1H NMR data of the few described isomers, except for the skeleton of the slovanolide *5a*[9,10] are distinctly different from those of the precursor of *1c*. The pronounced similarities of the NMR data (Table 1 and 2) indicated, that the precursor of *1c* is a slovanolide, too. The small amount of isolated compound prevented establishment of the locations of the acyl groups by chemical means. The assumption, however, that the acetic acid was esterified with O10, as is the case in all other guaianolides isolated from *Thapsia* species,[8] and that the allylic ester group was eliminated before the nonallylic ester group upon CI–MS, as is also the case in all other guaianolides isolated from *Thapsia* species,[8] imply that the precursor of *1c* has the structure of *5b*. The esterification pattern of *5b* has not previously been reported for a slovanolide.

Table 2. ^{13}C NMR data of compound *5a* and *5b*. Chemical shifts (δ values) of $CDCl_3$ solutions. Multiplicities are shown in parentheses.[a]

Carbon	Compound *5b*	Compound *5a*[b]
1	49.4[I]	49.3 (d)[I]
2	77.2[II]	77.4 (d)[II]
3	127.0	127.0 (d)
4	149.0	148.8 (s)
5	52.5[I]	53.0 (d)[I]
6	78.9[II]	78.7 (d)[II]
7	53.7[I]	53.2 (d)[I]
8	65.0	64.8 (d)
9	44.0	43.6 (t)
10	81.1	81.1 (s)
11	73.4	73.2 (s)
12	178.4	178.6 (s)
13	17.4[III]	17.4 (q)[III]
14	22.3[III]	22.3 (q)[III]
15	26.8[III]	26.4 (q)[III]

The signals due to the acyl moieties appear at the following δ values: *5b:* acetyl, 170.3, 22.8; α-metylbutanoyl, 176.3, 41.1, 16.5, 26.8, 11.6; butanoyl, 172.4, 36.4, 18.3, 13.7. *5a:* acetyl, 170.1 (s), 22.4 (q); α-metylbutanoyl, 175.2 (s), 41.1 (d), 16.3 (q), 26.2 (t), 11.5 (q); angeloyl, 167.4 (s), 127.6 (s), 138.0 (d), 15.7 (q), 20.4 (q).

[a] I, II, and III in the same vertical column: assignments may be interchanged.

[b] Taken from Ref. 12.

The ease by which *5b* was pyrolyzed into *1c* was illustrated by injection of a solution of the slovanolide into a gas chromatograph. Only one peak was detected, the retention time and mass spectrum of which were identical to those of *1c*. No detectable peak broadening indicated that the mechanism might be a retro-Prins[13]-like loss of pyruvic acid followed by an elimination of acetic, 2-methylbutanoic, and butanoic acid or vice versa.

From a biogenetic point of view, the presence of a slovanolide as well as *6* in *Thapsia garganica* is interesting, since this indicates that the unique α oriented C7–C11 bond in thapsigargin (*6*) is formed from a precursor having the generally observed β-oriented C7–C11 bond. The presence of this slovanolide also emphasizes the phytochemical relationship between the genera *Thapsia* and *Laserpitium* already pointed out by Holub.[14]

Experimental

General. The NMR spectra were recorded on a Bruker AM 500 spectrometer. The GC–MS was performed on a Finnigan 9500 gas chromatograph connected to a Finnigan 3500 D mass spectrometer; column: BP 5.25 m ×0.2 mm (SGE); injector 250 °C; oven 150–190 °C with a rate of 8 °C/min. The chemical ionization MS spectra were recorded on a VG 70–70 instrument equipped with a dual EI/CI ion source, using the direct inlet system, reagent gas isobutane, ion source temperature 220 °C. Column chromatography (CC) was performed over acid-washed silica gel (Merck 0.06–0.200 mm) standardized to contain 10 % water. Dry column chromatography was performed over silica gel for dry column chromatography, Woelm. HPLC was performed using a Waters 6000 A pump, a combined IR–UV detector (Knauer dual 61.00), and a prepacked LiChrosorb® RP 18 column, 8×250 mm, particle size 5 μm (Knauer). TLC was performed on Kieselgel 60 F 254 (Merck 5549) using toluene/ethyl acetate (4:1) as an eluent.

Plant material. The fruit of *T. garganica L.* (*Apiaceae*) were collected in early July 1976 by one of the authors (U.W.S.) in Algeria, ~35 km north of Constantine along road N3 to Skikda. A voucher specimen is being kept at the Department of Pharmacognosy, Royal Danish School of Pharmacy.

Isolation of pro-1,4-dimethylazulene (5b). The fruit was powdered and extracted twice with ethanol for 24 h. The ethanolic extract was concentrated and the residue partitioned between water and ethyl acetate. The organic layer was concentrated and the residue purified by CC using dichloromethane, to which increasing amounts of ethyl acetate (0–30 %) were added, as eluent. The fractions containing the component, which was visualized as a blue spot by heating the plates at 110 °C for 10 min, were concentrated and rechromatographed twice by CC using toluene/ethyl acetate (9:1) and toluene/ethyl acetate/methanol (18:1:1) as eluents. A final purification was performed by HPLC using methanol/water (1:1), containing 1 % of acetic acid, as an eluent. From 50 g of fruit, 4 mg of *5b* were isolated as a colourless amorphous powder. The compound was homogenous as evidenced by HPLC using methanol/water (1:1), to which was added 1 % of acetic acid, as an eluent. ^{1}H NMR and ^{13}C NMR data are given in Tables 1 and 2. Dominating ions in the chemical ionisation mass spectrum were [*m/z* values (rel.int.)]: 435(14), 333(55), 245(100), 199(23), 157(89), metastable peak at 180. GC–MS of a solution of *5b* afforded only one peak having the same retention time and mass spectrum as *1c*. The dominating peaks in the electron impact mass spectrum of *1c* were [*m/z* values (rel.int.)]: 156(63), 155(64), 141(100), 128(32), 115(61), cf. Refs. 5 and 16.

Preparation of the essential oil. Immediately before destillation, 100 g of fruit were suspended in water and triturated with an Ultraturrax® T45. Steam destillation was performed for 6 h according to the method of the European Pharmacopoeia for determination of volatile oil in vegetable drugs. The essential oil was dissolved in pentane and fractionated by dry/column chromatography (column 10 cm×5 cm).[15] The column was eluted with 150 ml of pentane, and the fraction concentrated. The ^{1}H NMR and MS spectra were in accordance with those described for *1c*.[5,16]

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