# 6. Dendryphiellin A, the First Fungal Trinor-eremophilane. Isolation from the Marine Deuteromycete *Dendryphiella salina* (SUTHERLAND) PUGH *et* NICOT

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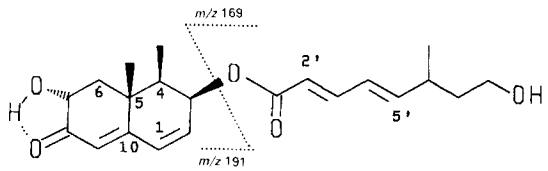
The novel metabolite dendryphiellin A (= (+)-(1*R*,2*S*,8a*R*)-1,2,6,7,8,8a-hexahydro-7-hydroxy-1,8a-dimethyl-6-oxonaphthalen-2-yl (6*R**,2*E*,4*E*)-8-hydroxy-6-methylocta-2,4-dienoate; (+)-**1**) is isolated from cultures of the marine deuteromycete *Dendryphiella salina*. There is no precedent in fungi for trinor-eremophilanes or for branched $C_9$ carboxylic acids, the two classes of compounds constituting (+)-**1**. The structure is secured by NMR spectroscopy and hydrolysis of (+)-**1** to give the side-chain moiety ((6*R**,2*E*,4*E*)-8-hydroxy-6-methylocta-2,4-dienoic acid (**2**)) intact, whilst the trinor-ermophilane moiety is decomposed. The absolute configuration at the trinor-eremophilane moiety is established from exciton coupling between the dienone and the diene-ester functions.

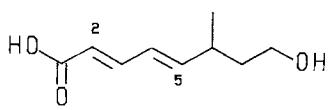
**1. Introduction.** – Although marine secondary metabolites are being actively searched in organisms belonging to many phyla [1] and have elicited a vast interest in related syntheses [2], the field of marine fungal metabolites is a most obscure area. This contrasts also with the wealth of studies devoted to terrestrial fungi, often resulting in the discovery of important metabolites [3].

As regards higher marine fungi, it is known that cultures of the basidiomycete *Halocyphina villosa* give the mildly antimicrobial and cytotoxic monoprenylated hydroquinone siccayne, previously isolated from the terrestrial deuteromycete *Helminthosporium siccans* [4a], whilst other higher marine fungi give free amino acids [4b]. Work with lower marine fungi has disclosed that cultures of the ascomycete *Leptosphaeria oraemaris* give the 2-aminohexose leptosphaerin [5a], which has also been synthesized [5b].

We report here the isolation from cultures of the marine deuteromycete *Dendryphiella salina* of a trinor-eremophilane esterified by a branched $C_9$ acid, a class of metabolites for which there is no precedent in fungi.




(+)-**1**




**2**

Table. *$^1$H-NMR Data for Dendryphiellin A ((+)-1) in $CD_3OD$*

| H-Atom[a]) | (+)-1 |
|---|---|
| H–C(1) | 6.46 (*d*, $J(1,2) = 9.8$) |
| H–C(2) | 6.26 (*dd*, $J(2,1) = 9.8$, $J(2,3) = 5.0$) |
| H–C(3) | 5.43 (*dd*, $J(3,4) = J(3,2) = 5.0$) |
| H–C(4) | 2.03 (*dq*, $J(4, CH_3–C(4)) = 7.0$, $J(4,3) = 5.0$) |
| $H_\alpha$–C(6) | 1.68 (br. *dd*, $J_{gem} = 12.7$, $J(6\alpha,7) = 13.2$, $J(6\alpha, CH_3–C(5))$ small) |
| $H_\beta$–C(6) | 2.37 (*dd*, $J_{gem} = 12.7$, $J(6\beta,7) = 5.5$) |
| H–C(7) | 4.41 (*dd*, $J(7,6\alpha) = 13.2$, $J(7,6\beta) = 5.5$) |
| H–C(9) | 5.86 (*s*) |
| $CH_3$–C(5) | 1.41 (br. *s*, $J$ ($CH_3$–C(5), 6α) small) |
| $CH_3$–C(4) | 1.06 (*d*, $J$ ($CH_3$–C(4),4) = 7.0) |
| H–C(2′) | 5.87 (*d*, $J(2',3') = 15.2$) |
| H–C(3′) | 7.28 (*dd*, $J(3',2') = 15.2$, $J(3',4') = 10.8$) |
| H–C(4′) | 6.29 (*dd*, $J(4',5') = 15.4$, $J(4',3') = 10.8$) |
| H–C(5′) | 6.10 (*dd*, $J(5',4') = 15.4$, $J(5',6') = 8.1$) |
| H–C(6′) | 2.47 (*m*, $J(6', CH_3–C(6')) = J(6',7') = 6.8$, $J(6',5') = 8.1$) |
| 2H–C(7′) | 1.59 ($A_2$ of $A_2XY$ as a *ddd*, $J(A_2,X) = J(A_2,Y) = J(7',6') = 6.8$) |
| 2H–C(8′) | 3.56 and 3.54 ($XY$ of $A_2XY$, $J(XY) = 10.5$, $J(X,A_2) = J(Y,A_2) = 6.8$) |
| $CH_3$–C(6′) | 1.07 (*d*, $J(6',CH_3–C(6')) = 6.8$) |
| HO–C(7) | 1.98 (br. *s*) |

[a]) Eremophilane numbering.

**2. Results and Discussion.** – The new metabolite, dendryphiellin A ((+)-**1**), is a colourless oil of high optical activity. Its structure and relative configuration is established by spectral data.

$^{13}$C-NMR spectra reveal 21 C-resonances (*Exper. Part*) which are consistent with a δ-substituted α,β,γ,δ-unsaturated ester, a β,β-disubstituted α,β-enone, a 1,2-disubstituted C=C function, and a series of signals in the region of $sp^3$ C-atoms comprising 1 $CH_2$–O *t*, 2 CH–O *d*, 1 *s*, 2 *d*, 2 *t*, and 3 *q*. From these data, the molecular composition of dendryphiellin A can be either $C_{21}H_{26}O_4$, if there is an intramolecular ether bridge, or $C_{21}H_{28}O_5$, if there are 2 OH functions.

The presence of the $CH_2$–O and CH–O functions are confirmed by the $^1$H-NMR spectrum (*Table*) of (+)-**1** (3.54, 3.56 ($XY$ of $A_2XY$, 2 H–C(8′)); 4.41 (*dd*, H–C(7))). Whilst the corresponding patterns might result from an intramolecular CH–O–$CH_2$ bridge, other evidence suggests the $CH_2$–OH and CH–OH functions instead. Thus, though the $^1$H-NMR spectrum has been recorded in a H/D-exchanging medium ($CD_3OD$), the br. *s* (1 H) at 1.98 ppm undergoes a shift on temperature change, indicating an OH group. Failure of exchange of this OH group with the solvent suggests a strong intramolecular H-bond. The high polarity of the compound (see *Exper. Part*) is in agreement with the presence of OH rather than ether groups, and these observations imply that an OH function is part of a cycle with the enone carbonyl group as the H-bond acceptor.

Detailed $^1$H,$^1$H decouplings and COSY maps show that the primary OH function lies at the $CH_2$ terminus of a CH=CHCH=CHCH($CH_3$)$CH_2CH_2$ chain. The other terminus is attached to the ester carbonyl group (see $^{13}$C-NMR), as indicated by a $^{13}$C,$^1$H correlation and chemical-shift data for the C- and H-atoms of the 4 adjacent CH groups along this chain ($^{13}$C-NMR: 124.71 (C(α)); 147.29 (C(β)); 128.28 (C(γ)); 155.55 (C(δ)) [6]). Further support is given by a 2D long-range correlation between the ester C=O and H–C(β).

The presence of such a side chain is confirmed by the MS (*Exper. Part*) that shows no $M^{+\cdot}$ but a weak peak at *m/z* 169 and an intense at *m/z* 191. The former stems from the side chain and the latter from the remaining part of (+)-**1**. Full confirmation was obtained by recovering the unaltered side chain as the carboxylic acid **2** from the base hydrolysis of (+)-**1**[1]).

The portion of (+)-**1** after removal of the side chain has the composition $C_{12}H_{15}O_2$ implying 5 unsaturations. The NMR spectra (*Exper. Part* and *Table*) indicate 1 carbonyl and 2 C=C groups suggesting a bicyclic

[1]) Under the hydrolytic and workup conditions, the bicyclic moiety of dendryphiellin A decomposed into a multitude of products, none of which could be isolated in sufficient amount for a structural study.

structure. $^1H,^1H$ Decouplings, COSY maps, and differential NOE effects support the bonding sequence CH(9)=C(10)–CH(1)=CH(2)–CH(3)–CH(4)($CH_3$)–C(5)($CH_3$)–$CH_2$(6)–CH(7)(OH) (eremophilane numbering, see *Formula* (+)-**1**). H–C(9) is related to C(1) by a 2D long-range $^{13}C,^1H$ correlation and to H–C(1) by a +15% differential NOE effect. In turn, $CH_3$–C(5) has a long-range $^1H,^1H$ coupling with $H_\alpha$–C(6) and a long-range 2D $^{13}C,^1H$ correlation with C(6). Moreover, there are differential NOE effects of +14% between $CH_3$–C(5) and $H_\beta$–C(6) and of +2.5% between $CH_3$–C(5) and $CH_3$–C(4), while the CH(1)=CH(2)–CH(3) group is immediately located between C(10) and C(4) on the basis of the $^1H$-NMR data in the *Table*. At this point, and on the additional basis of a long-range 2D $^{13}C,^1H$ correlation between H–C(7) and C(9), we are prompted to place the known enone carbonyl group so as to bridge C(7) and C(9) of the above bonding sequence. Moreover, to account for both $\beta,\beta$-disubstitution at the enone group and a quaternary C(5), C(5) and C(10) have to be joined together.

The resulting structure (+)-**1** allows a stereochemically favourable, and thus strong, H-bond between OH–C(7) and the enone carbonyl group. The arrangement of the chromophores is in accordance with the fact that the UV absorption band of (+)-**1** at 272 nm is wide enough to account for the non-coincident enonic and unsaturated-ester absorption bands, as suggested by *Woodward*'s calculation [7].

The relative configuration of (+)-**1** is supported by the following observations. The $\beta$ configuration for both the side chain and the $CH_3$–C(5) group, *i.e.* the proximity of $CH_3$–C(5) to O–C(=O), are implied by a strong deshielding of $CH_3$–C(5) (1.41 ppm). This is also supported by a +3% differential NOE effect between $CH_3$–C(5) and H–C(2'). Moreover, the W relationships between either H–C(3) and H–C(1) or $CH_3$–C(5) and $H_\alpha$–C(6)[2]) demand $\beta$ configuration for both the side chain and $CH_3$–C(5) and $\alpha$ configuration for OH–C(7). The equatorial $\beta$ position for $CH_3$–C(4) is supported by a +2.5% differential NOE effect between this group and $CH_3$–C(5) and by a +6% NOE effect between H–C(4) and $H_\alpha$–C(6)[3]).

As regards the ester chain, the all-*trans* configuration rests on large $^1H,^1H$ coupling (*J ca.* 15 Hz) for H–C(4')/H–C(5') and H–C(2')/H–C(3').

The absolute configuration at the bicyclic system as depicted in (+)-**1** is established from internal exciton coupling of the enone with the unsaturated-ester chromophore giving rise to a positive first band and a negative second band [8].

Dendryphiellin A, apparently, is a trinor-eremophilane, the isopropyl group at C(7) of eremophilane which is a most common feature of terrestrial fungi [3] being replaced by an alcoholic group. Such a truncated eremophilane skeleton is unprecedented in fungal metabolites. To our knowledge, truncated eremophilanes lacking the isopropyl group have only been isolated from the terrestrial herb *Senecio humillimus* (Compositae) [9a] and from the Chinese terrestrial plant *Nardostachys chinensis* (Valerianaceae) [9b].

We are not aware of any truncated eremophilanes of marine origin. Even intact eremophilanes of marine origin are rare. A representative has been found in the soft coral *Lemnalia africana* collected around the Western Caroline Islands [9c].

The ester side chain of (+)-**1** which (with H–C(8') instead of OH–C(8')) is part of saponins of the terrestrial plant *Acer negundo* (Aceraceae) [10] is also of a structural type that has never been encountered before in fungi. The closest analogue is a $CH_3CH(OH)C(OH)(CH_3)$ chain of clear mevalonic origin in metabolites of the terrestrial fungus *Candida tenuis* [11]. In the absence of biosynthetic studies, the origin of the side chain of (+)-**1** can be conceived either along the mevalonic-acid pathway, with demethylation at C(2') and shift of a double bond, or along the fatty-acid pathway, with methylation at C(6'). In view of the ability of *D. salina* in degrading eremophilanes, we favour the mevalonic-acid hypothesis also for the side chain of (+)-**1**.

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[2]) With $H_\alpha$–C(6) in the axial position to account for *trans*/diaxial coupling with H–C(7) (*Table*) which has a +20% differential NOE effect with $CH_3$–C(5).

[3]) The peaks at *m/z* 170 and 190 have different origin. They originate from the elimination of the acid **2**.

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## Experimental Part

1. *General.* All evaporations were carried out at reduced pressure. Flash chromatography: *Merck RP-18 LiChroprep* 40–65 μm. HPLC: *Merck-LiChrosorb CN* (7 μm). Reverse-phase HPLC: *Merck-LiChrosorb RP18* (7 μm); columns 25 × 1 cm; solvent flux 5 ml/min; monitoring by UV at 245 nm. Polarimetric data: *JASCO-DP-181* digital polarimeter. UV ($\lambda_{max}$ in nm, $\varepsilon$ in $mol^{-1}$ l $cm^{-1}$): *Perkin-Elmer-Lambda-3.* CD: *JASCO J-500 A.* NMR: *Varian-XL-300* ($\delta$ (ppm) rel. to internal $Me_4Si$ (= 0 ppm); probe temp. 21°). $^1$H-NMR at 300 MHz, *J* in Hz, couplings from double irradiations and COSY [12] experiments. The NOE experiments were carried out with 8 s of preirradiation. $^{13}$C-NMR at 75.43 MHz, multiplicities from DEPT [13], chemical-shift assignments from $^{13}$C,$^1$H correlations [14]. EI-MS (*m/z* (%)): home-built spectrometer based on the *ELFS-4-162-8-Extranuclear* quadrupole [15].

2. *Culture and Isolation. D. salina* (SUTHERLAND) PUGH *et* NICOL was cultured on Cornmeal Disk *(Cornmeal)*. A culture of 10 l was lyophylized and the residue extracted first with AcOEt and then with MeOH. The MeOH phase was defatted with hexane and then subjected to reverse-phase flash chromatography with a gradient of MeOH/$H_2O$. Of a total of 11 fractions, the 9th fraction was evaporated and the residue subjected to reverse-phase HPLC with MeOH/$H_2O$ 6:4 collecting 3 fractions. Fractions 1 and 2 were evaporated and the combined residues subjected to HPLC with hexane/EtOH/AcOH 92:8:2 to give pure (+)-**1** (0.006 g).

3. *Dendryphiellin A* (= *(+)-(1R,2S,8aR)-1,2,6,7,8,8a-Hexahydro-7-hydroxy-1,8a-dimethyl-6-oxonaphthalen-2-yl (6R*,2E,4E)-8-Hydroxy-6-methylocta-2,4-dienoate;* (+)-**1**). Colourless oil. $[\alpha]^{20}$ ($\lambda$) = +572.1° (589), +607.7° (577), +716.0° (546), +1596.1° (435; *c* = 0.31, abs. EtOH). UV (EtOH): 272 (37100). CD (EtOH, 4.33 × $10^{-5}$ M; optical path 1 cm; sensitivity 5; $\lambda$ in nm (elongation in cm, $\Delta\varepsilon$ in $mol^{-1}$ l $cm^{-1}$): 252 (–5.1, –17.8), 279 (+13.6, +47.6). $^1$H-NMR ($CD_3OD$; irradiated proton(s) ($\delta$)→% NOE effect on the observed proton(s)): 1.41→+20% on H–C(7), +10% on $H_\beta$–C(6), + 2% on $CH_3$–C(4), + 3% on H–C(2'); 1.68→6% on H–C(4); 2.03→+16% on H–C(3), +1% on $CH_3$+C(4); 5.43→+6% on H–C(4), +8% on H–C(2); 5.86 and 5.87→+16% on H–C(1), +9% on H–C(4'), besides +5% on the low-field and –3% on the high-field signals of H–C(3'), which arise from a strongly coupled three-spin system [16]; 6.46→4% on H–C(2), 15% on H–C(9). $^{13}$C-NMR ($CD_3OD$): 131.54 (*d*, C(1)); 134.10 (*d*, C(2)); 69.94 (*d*, C(3)); 42.46 (*d*, C(4)); 38.82 (*s*, C(5)); 44.54 (*t*, C(6)); 70.95 (*d*, C(7)); 201.35 (*s*, C(8)); 119.96 (*d*, C(9)); 163.93 (*s*, C(10)); 19.46 (*q*, *C*–C(5)); 10.43 (*q*, *C*–C(4)); 168.16 (*s*, C(1')); 124.71 (*d*, C(2')); 147.29 (*d*, C(3')); 128.28 (*d*, C(4')); 151.55 (*d*, C(5')); 35.07 (*d*, C(6')); 40.12 (*t*, C(7')); 60.74 (*t*, C(8')); 20.37 (*q*, *C*–C(6')). MS: 191 (13), 190 (35), 176 (32), 175 (82), 173 (6), 170 (3), 169 (1), 152 (8), 148 (37), 147 (93), 107 (54), 91 (100), 79 (64).

4. *(6R*,2E,4E)-8-Hydroxy-6-methylocta-2,4-dienoic Acid* (**2**). A soln. of (+)-**1** (3.6 mg, 0.01 mmol) in 1 ml of 3% KOH/MeOH was stirred at r.t. for 2 h. The mixture was neutralized and evaporated and the residue subjected to TLC with $Et_2O$ to give **2** (1 mg). $R_f$ 0.35. $[\alpha]^{20}(\lambda)$ = +53.9° (589), +55.0° (577), +66.0° (546), +123.2° (435), +270.6° (365; *c* = 0.09, MeOH). UV (MeOH): 259 (20800). $^1$H-NMR ($CD_3OD$): 7.26 (*dd*, *J*(3,2) = 15.3, *J*(3,4) = 10.7, H–C(3)); 6.25 (*dd*, *J*(4,3) = 10.7, *J*(4,5) = 15.3, H–C(4)); 6.08 (*dd*, *J*(5,4) = 15.3, *J*(5,6) = 8.0, H–C(5)); 5.81 (*d*, *J*(2,3) = 15.3, H–C(2)); 3.56, 3.55 (*XY* of $A_2XY$, *J*(*X*,*Y*) = 10.4, *J*(*X*,$A_2$) = *J*(*Y*,$A_2$) = 6.8, 2H–C(8)); 2.47 (*m*, *J*(6,5) = 8.0, *J*(6, $CH_3$–C(6)) = *J*(6,7) = 6.8, H–C(6)); 1.58 ($A_2$ of $A_2XY$ as a *q*, *J*($A_2$,*X*) = *J*($A_2$,*Y*) = *J*(7,6) = 6.8, 2 H–C(7)); 1.07 (*d*, *J*($CH_3$–C(6),6) = 6.8, $CH_3$–C(6)). MS: 152 (13, $M^{+\cdot} - H_2O$), 135 (6), 134 (4), 107 (70), 97 (52), 91 (78), 79 (100), 55 (44), 45 (65).

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