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Relative Contributions to Antitumoral Activity of Lipophilic vs. Polar Reactive Moieties in Marine Terpenoids

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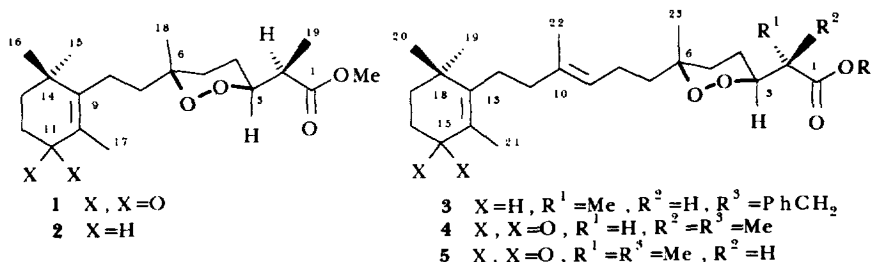
Abstract: Marked cytotoxicity of norsesterterpenoids **3** and **4** vs. inactivity or low activity of the respective norditerpenoid analogues **2** and **1** towards KB tumoral cell lines suggest that the length of the lipophilic chain is an important factor while, *per se* the reactive peroxide functionality is not. Moreover, lower bioactivity towards the same cells of **1** than **2**, and comparable activities of **3-5**, show the unimportance *per se* of the highly electrophilic enone moiety. A strict need of molecular tailoring, so that weapons may reach the target in adaptive phenomena, may be at the basis of natural selection of these compounds and suggests strategies for the design of safer agents against cancer. © 1997 Elsevier Science Ltd.

Although the selectivity of drugs has much improved since the first discriminative synthetic antiparasitic agents of the late nineteenth century,[1] target-specific agents that do not elicit unwanted side effects remain ideal drugs. This is particularly the case of antineoplastic agents which typically cause a variety of harmful side effects and have low therapeutic index.[2] We are examining natural products in this perspective, since it is from their immense chemical diversity that innovative drugs are still largely expected. Results presented here for terpenoids **1-5**, extracted from the sponge *Diacarnus levii* (Kelly-Borges and Vacelet)[3] (Hadromerida, Latrunculiidae) collected



(731M/R1528) in January 1992 along the outer reef in front of Noumea, New Caledonia, at depths 6-15 m suggest a nature's effective strategy with these adaptive compounds that has no precedent in the finely tuned combination of polar reactive and lipophilic inert moieties.

The sponge was deep frozen and freeze-dried. The powder, 900 g, was EtOH extracted, filtered, the filtrate evaporated, added of H_2O and CH_2Cl_2 extracted and the organic extract, dried over Na_2SO_4, was evaporated to give 6.5 g of residue which was subjected to RP-18 FC with $MeOH/H_2O$ gradient. A portion, 0.2 g,

Table 1. NMR spectra of 3,6-epidioxy nordi- (1-2) and norsesterpenes (3-5) from *D. levii* (in $CDCl_3$[a] unless otherwise stated)

	1		2		3[b]		4		5	
H/C	¹H	¹³C	¹H	¹³C	¹H	¹³C	¹H	¹³C	¹H	¹³C
1		174.13,s		174.25,s		173.68,s		174.25,s		174.34,s
2	2.67, br.dq, 7.0	42.45,d	2.65, br.dq, 7.0	42.95,d	2.65,d	42.86,d	2.66,br.dq	42.91,d	2.59,dq	42.80,d
3	4.10, m[c]	81.19,d	4.13,ddd, 9.0,7.5,3.3	81.30,d	4.27,m[d]	81.63,d	4.12,m[e]	81.38,d	4.22,m[f]	81.73,d
4	1.70,m	23.25,t	1.68,m	21.68,t	1.68,m	22.69,t	1.70,m	23.43,t	1.70,m	22.78,t
5	1.66,m	31.76,t	1.65,m	31.81,t	1.67,m	32.2,t	1.68,m	31.98,t	1.68,m	32.41,t
6		79.85,s		80.35,s		80.06,s		80.05,s		80.00,s
7	1.68,m	37.75,t	1.68,m	39.00,t	1.48,m	40.18,t	1.48,m	39.60,t	1.50,m	40.16,t
8	2.22,m, 2.26,m	24.13,t	2.02,m	21.76,t	2.02,m	21.57,t	2.03,m	21.66,t	2.03,m	21.68,t
9		163.95,s		136.36,s	5.10,br.tq, 7,1	123.23,d	5.15,br.tq 7.1,1.1	124.47,d	5.15,br.tq, 7.1,1.1	124.62,d
10		131.12,s		127.30,s		136.42,s		135.02,s		135.02,s
11		198.63,s	1.88,m	32.74,t	2.00,m	40.18,t	2.07,m	38.38,t	2.07,m	38.46,t
12	2.42,m	34.21,t	1.55,m	19.47,t	2.02,m	27.80,t	2.28,m	30.00,t	2.28,m	30.04,t
13	1.76,m	37.44,t	1.41,m	39.01,t		137.05,s		164.69,s		164.45,s
14		36.47,s		35.04,s		126.90,s		130.83,s		130.94,s
15	1.12,s	26.91,q	0.97,s	28.60,q	1.90,br.t,6.5	32.72,t		199.07,s		198.78,s
16	1.13,s	26.91,q	0.98,s	28.57,q	1.57,m	19.53,t	2.45,m	34.25,t	2.45,m	34.29,t
17	1.72,s	11.39,q	1.57,s	19.73,q	1.41,m	39.81,t	1.80,m	37.35,t	1.79,m	37.52,t
18	1.30,s	20.72,q	1.32,s	20.45,q		34.95,s		36.37,s		36.40,s
19	1.23,d,7.0	13.62,q	1.25,d,7.0	13.61,q	0.99,s	28.59,q	1.16,s	26.90,q	1.16,s	26.98,q
20					0.99,s	28.59,q	1.16,s	26.90,q	1.16,s	26.98,q
21					1.60,s	19.80,q	1.77,s	11.53,q	1.77,s	11.50,q
22					1.63,br.s	15.98,q	1.66,d,1.1	15.93,q	1.66,d,1.1	15.91,q
23					1.31,s	20.30,q	1.30,s	20.66,q	1.32,s	20.41,q
24					1.17,d,7.5	12.79,q	1.25,d,7	13.59,q	1.15,d,7.2	12.81,q
MeO	3.67,s	51.74,q					3.70,s	51.87,q	3.70,s	51.82,q

[a] ^1H at 299.94 MHz, ^{13}C at 75.43 MHz, δ with respect to internal Me_4Si=0, 20 °C; [b] δ_H 5.14 and 5.16 (AB,12.0,CH_2Ph), 7.35 (m, Ph); δ_C 66.38 (t,CH_2Ph), 128.04((d),128.08(d),128.47(d),135.96(s),Ph): [c] δ_H 4.33, td, 8.5,7.5,4.0 in C_5D_5N at 60 °C; [d] δ_H 4.40, td, 8,8,5 in C_5D_5N at 70 °C; [e] δ_H 4.34, ddd, 9.0,7.5,3.3 in C_5D_5N at 40 °C; [f] δ_H 4.40, td, 8,8,5 in C_5D_5N at 70 °C.

of the fraction (1.72 g) eluted with MeOH was subjected to SiO_2 TLC with 4:1 pet. ether/Et_2O to give methyl 2-epinuapapuanoate (**2**) (R_F 0.75, 26 mg)[4] and a mixture of carboxylic acids (R_F 0.25, 78 mg) which were esterified in 70% yield with *p*-DMAP/DCC/benzyl-alcohol in benzene and from which Si60 HPLC with 49:1 hexane/AcOEt (here and subsequently 5 ml/min) allowed us to obtain 2-epimukubilin benzyl ester (**3**) (t_R 15 min,

23 mg).[5] The FC fraction, 0.33 g, eluted with 9:1 MeOH/H_2O was subjected to CN FC with hexane/Et_2O gradient elution; the initial fractions (0.12 g), subjected to Si60 HPLC with 4:1 hexane/AcOEt, gave methyl diacarnoate A (**1**) (t_R 13 min, 38 mg).[6] Subsequent CN FC fractions, treated with carbonyldiimidazole in MeOH, gave a mixture of methyl esters (0.14 g, 74%) that on HPLC as above afforded methyl prenyldiacarnoate A (**4**) (t_R 9 min, 6 mg)[7] and methyl 2-epi-prenyldiacarnoate (**5**) (t_R 11.5 min, 11 mg).[8] Two cycles for **1** rest on the composition $C_{20}H_{32}O_5$ deduced from NMR atom count and HR-MS on the fragment ion for loss of the side chain from C3, on account of the presence of an ester group and an α-methyl-β-alkyl enone group (Table 1). The position for the latter finds evidence in couplings: $H_2$12 with both C11-O and C13, the gem-dimethyl groups with C13, C14 and C9, and $H_2$8 with C9, C10 and C14. Coupling of $H_2$8 with C7 and of $H_3$18 with C7, C6 and C5 supports the side chain until inside the dioxane ring.[9] Finally, coupling of H-2 with C1, C3 and C19 allowed the complete elucidation of the structure. According to widely accepted empirical rules,[9b] δ_{H19} = 1.23 is in the correct range for *threo* configuration at C2-C3 while δ_{C18} = 13.62 supports the axial position of the methyl group at C6. Structure **2** was secured from spectra quite similar to **1**, except for replacement of the carbonyl by a methylene group. *Threo* C2-C3 configuration rests on δ_H 1.23 for $H_3$19, while axial C18 is supported by δ_H 1.30.[9] Spectral data for **3** recall those for muqubilin,[9a] and were confirmed by HBMC, except for δ_H 1.17 for $H_3$24 suggesting *erythro* configuration at C2-C3, and thus assigning the compound as the new 2-epimuqubilin. MS and NMR data (Table 1) similarly support the structures **4** for methyl prenyldiacarnoate and **5** for methyl 2-epi-prenyldiacarnoate. The configurations at C-2 and the epidioxy moiety are preliminarily drawn by comparison with literature chiroptical data.[9]

The results of cytotoxicity assays of these compounds on KB tumoral cell lines reveal (Table 2) that the

Table 2. Cytotoxicity of compounds **1-5** from the sponge *Diacarnus levii* towards KB tumoral cell lines*

Compound	IC_{50} (μg ml^{-1})
1	inactive
2	>>20
3	1.0
4	3.3
5	0.9

*KB tumour cell lines for these assays were grown as a unicellular film in MEM medium containing Earle's salts and L-glutamine (Sigma M 0268) added of 10% foetal bovine serum (Sigma F2442). After trypsin treatment, exponentially growing cells were seeded (10,000 cells/ml) in 96-microwell plates and exposed to the test compounds at various concentrations one hour after seeding. The cells were then incubated for 3 days, neutral red was incorporated, and, after 24 h, inhibition of cell growth was evaluated by discarding dead cells on rinsing the plates with PBS and hydrolysing the surviving coloured cells with SDS. The optical density at 540 nm, which is representative of the number of surviving cells, was then measured on a Titertek Uniskan apparatus. The antiproliferative activity of the test compounds was calculated from dose-response curves and reported as IC_{50} from experiments carried out as above in triplicate at different times on the same plate for different compounds.

smallest and less functionalised member, **2**, has only negligible activity. However, insertion of a prenyl unit in the central portion of the molecule makes **3** far more active than **2**, actually in the range of potentially useful drugs (Table 2). This shows the importance of the lipophilic portion of the molecule[10] and the unimportance, *per se*, of the reactive peroxide functionality. Moreover, replacement of the allylic methylene in either **2** or **3** by a carbonyl group to get **1** and **5**, respectively, did not improve the cytotoxicity. Actually, **1** has undetectable activity. This shows the unimportance, *per se*, of the highly electrophilic α,β-unsaturated functionality, which is otherwise a much estimated enhancing factor in the design of reactive antitumoral agents. Finally, similar cytotoxicity of **4** and **5** shows the unimportance of the relative configuration at C-2.

The whole body of these data suggests that marked cytotoxicity of norsesterterpenoids **3-5**, and negligible

cytotoxicity of **1** and **2** result from recognition of the molecules *in toto*. The superior character of norsesterterpenoids (**3-5**) may be rationalized along two alternative lines, both implying the importance of the elongation of the molecule by an extra prenyl unit. The effect of the longer chain may be twofold: it allows the norsesterterpenids to cross cell membranes (lipophilicity effect) and it makes them to fit receptors (size effect). Judging from the similar bioactivities of **3-5**, the α,β-enone group does not appear to be used for its high electrophilicity. The role played by the peroxide group is difficult to judge since this group can not be removed without changing the nature of the molecule and because literature terms of comparison for this functionality are scarce, except for antimalarial agents.[11] The "lipophilicity effect" might be a nature's trick to convey this highly reactive functionality to the target. This could be at the basis of natural selection of these compounds.

Our data reflect the combination of all the above and other factors involved in bioassays with whole cells. In order to disentangle these factors, mechanism-oriented bioassays are planned on the above products. At any event, the structure-activity relationships reported here suggest strategies for the structural modification of pharmacologically promising compounds[12] towards safer drugs for cancer chemotherapy.

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4. Data of **2**: $[\alpha]_D^{25}$ = -33.0, (*c* 0.4, $CHCl_3$); EI-MS *m/z* (%) 338.
5. Data of **3**: $[\alpha]_D^{25}$ = +20.2, (*c* 0.29, $CHCl_3$); EI-MS *m/z* (%) 482 (1), 319 (3), 181 (15), 91 (100).
6. Data of **1**: $[\alpha]_D^{25}$ = -18.2, (*c* 0.76, $CHCl_3$); EI-MS *m/z* (%) 352 (1), 265 (1), 165 (31), 137 (27), 99 (24), 43 (100); HR-EI-MS *m/z* 352.224 ± 0.005, [$C_{20}H_{32}O_5$] calc. 352.225.
7. Data of **4**: $[\alpha]_D^{25}$ = -26.4, (*c* 0.30, $CHCl_3$); EI-MS *m/z* (%) 420 (1), 33 (8), 181 (4), 165 (21), 153 (18), 43 (100); HR-EI-MS *m/z* 420.286 ± 0.005, [$C_{25}H_{40}O_5$] calc. 420.288.
8. Data of **5**: $[\alpha]_D^{25}$ = +19.5, (*c* 0.28, $CHCl_3$); EI-MS *m/z* (%) 420 (1), 333 (7), 181 (5), 165 (21), 153 (18), 43 (100); HR-EI-MS *m/z* 420.287 ± 0.005, [$C_{25}H_{40}O_5$] calc. 420.288.
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