New Types of Potentially Antimalarial Agents: Epidioxy-Substituted Norditerpene and Norsesterpenes from the Marine Sponge *Diacarnus levii*

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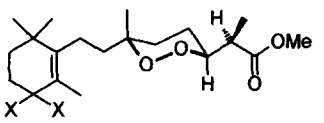
Natural free carboxylic acids from the hadromerid sponge *Diacarnus levii* (Kelly-Borges and Vacelet) were esterified to yield the new cyclic norditerpene peroxides *ent*-muqubilin benzyl ester (= (α*R*,3*S*,6*R*)-α,6-dimethyl-6-[(*E*)-4-methyl-6-(2,6,6-trimethyl-cyclohex-1-en-1-yl)hex-3-enyl]-1,2-dioxan-3-acetic acid benzyl ester; **6**), diacarnoate B methyl ester (= (α*S*,3*S*,6*R*)-α,6-dimethyl-6-{2-[(4a*S*,8a*S*)-3,4,4a,5,6,7,8,8a-octahydro-3-oxo-2,5,5,8a-tetramethylnaphthalen-1-yl)ethyl}-1,2-dioxan-3-acetic acid methyl ester; **9**), and deoxydiacarnoate B benzyl ester (= (α*S*,3*R*,6*R*)-α,6-dimethyl-6-{2-[(4a*S*,8a*S*)-3,4,4a,5,6,7,8,8a-octahydro-2,5,5,8a-tetramethyl-1-naphthalenyl]ethyl}-1,2-dioxan-3-acetic acid benzyl ester; **10**), which were isolated following extensive chromatography. The relative configuration of the peroxide/α-methylacetate moiety of **6**, **9**, and **10** was directly determined from their NMR spectra. The absolute configurations of the peroxide/α-methylacetate moiety was deduced from comparative ^{1}H-NMR data of the (*S*)- and (*R*)-phenylglycine methyl ester derivatives **7** and **8** as well as **11**/**13** and **12**/**14**, all obtained from a mixture of the precursors of **3**, **6**, and **10**. The absolute configuration at the carbobicyclic moiety of enone **9** and of **10**, is identical, as established by chemical interconversion, **9** and **10** belong to the normal labdane series according to empirical CD rules, applied either directly to **9** or to the parent (+)-sclareolide-derived enone **20**. In contrast, molar rotation additivity rules suggest the *ent*-labdane configuration for **9** and **10**. The epidioxides **1**–**3**, **6**, and **10** proved active *in vitro* against the malaria parasite *Plasmodium falciparum*; especially the previously isolated methyl 3-epinuapapuanoate (**2**) was active against a chloroquine-resistant strain, and this with a good security index.

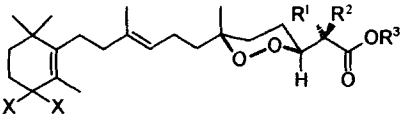
1. Introduction. – Malaria is endemic in Mexico, most of South America and Africa, the Middle East, Indochina, and Indonesia. Each day, 0.8–1.4 million people become sick, and 4800–7200 children die from malaria, to which pregnant women are not immune [1]. Prospects about malaria are of a reemerging disease that may threaten even traditionally non-endemic areas and areas that had been freed of, or never touched by, this plague [2]. The multiform nature of this illness, with variability and acquired resistance of the responsible parasite, cause much concern and demand for new drugs.

To this concern, we have further investigated a hadromerid sponge collected in New Caledonia, *Diacamus levii* (Kelly-Borges and Vacelet), which had already provided a series of epidioxy-terpenes with cytotoxic activity on human tumour cell lines. These included epidioxy-norditerpenes, such as methyl diacarnoate A (**1**) and methyl 3-epinuapapuanoate (**2**), and epidioxy-norsesterterpenes, such as 2-epimukubulin benzyl ester (**3**), methyl prenyldiacarnoate A (**4**), and methyl 2-epi-prenyldiacarnoate A (**5**), all isolated as esters of the natural free carboxylic-acid forms [3]. We now isolated from this sponge related epidioxy-norsesterterpenes which vary in chiral properties with respect to

analogues isolated from other marine sponges, and which show substantial *in vitro* activity against chloroquine-resistant *Plasmodium falciparum* strains, having thus potential as new antimalarial leads.

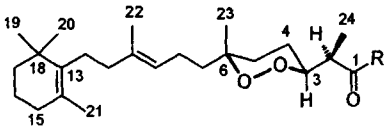


1 X,X=O
2 X=H

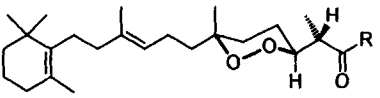


3 X=H, R^1=Me, R^2=H, R^3= $PhCH_2$
4 X,X=O, R^1=H, R^2=R^3=Me
5 X,X=O, R^1=R^3=Me, R^2=H

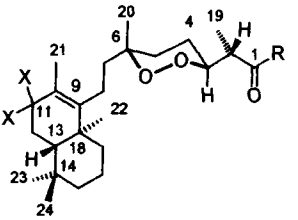
2. Results and Discussion. – Spectral data for the new *ent*-muqubilin benzyl ester (**6**; *Exper. Part*) match those for muqubilin [4], except for the data due to esterification by benzyl alcohol and for negative optical rotation. In particular, the *q* at δ(C) 20.68 supports the axial position for Me(23), while the *d* at δ(H) 1.28 for Me(24) indicates *threo* configuration at C(2)–C(3) [5].



6 R=OCH_2Ph
7 R=(*S*)-NHCH(Ph)CO_2Me
8 R=(*R*)-NHCH(Ph)CO_2Me



13 R=(*S*)-NHCH(Ph)CO_2Me
14 R=(*R*)-NHCH(Ph)CO_2Me



9 X,X=O, R=OMe
10 X=H, R=OCH_2Ph
11 X=H, R=(*S*)-NHCH(Ph)CO_2Me
12 X=H, R=(*R*)-NHCH(Ph)CO_2Me

Spectral data for the new deoxydiacarnoate B benzyl ester (**10**) (*Exper. Part*) recall mycaperoxide B [6a] and related products [6b]. In particular, the *q* at δ(C) 20.09 supports an axial Me(20) and the *d* at δ(H) 1.19 for Me(19) indicates *erythro* configuration at C(2)–C(3) [5]. Spectral data for the new methyl ester **9** are similar to those of **10** except for the enone group.

The determination of the absolute configuration of these compounds was next addressed. Attempts at chemical correlation of **10** with mycaperoxide B of known absolute configuration [6a] failed: compound **10** did not add H_2O in the presence of *Amberlyst-15*, while epoxides obtained from **10** with 3-chloroperbenzoic acid decomposed in an uncontrolled fashion during attempts at reduction to mycaperoxide B. However, conversion of **10** to natural **9** by oxidation of the allylic CH_2(11) group established the same absolute configuration for the two compounds. For the determination of the configuration of **3** and **6**, as well as of the peroxide and α-methylacetate moieties of **10**, we chose the phenylglycine methyl ester method developed for α-chiral carboxylic acids [7], after

various other unsuccessful attempts[1]). Amidation of the mixture of the free carboxylic-acid precursors of **3**, **6**, and **10** with, in turn, the enantiomers of phenylglycine methyl ester, allowed us to separate pure **7** and **8**. The $\Delta\delta = \delta(S) - \delta(R)$ values (*Exper. Part*) pointed to the configuration (2*R*,3*S*,6*R*) for **6** according to the proposed model [7] (*Fig.*, **A**). In contrast, only the mixtures of amides **11**/**13** and **12**/**14** could be isolated. However, the relevant $\Delta\delta = \delta(S) - \delta(R)$ values could be deduced from the spectra (*Exper. Part* and *Fig.*, **B**), which allowed the assignment of the configuration (2*S*) to both **3** and **10** according to the proposed model [7]. Therefore, in virtue of the assigned relative configuration as well as of the interconversion of **10** into **9**, the (2*S*,3*S*,6*R*)-configuration was attributed to the peroxide and α-methylacetate moiety of both **9** and **10**.

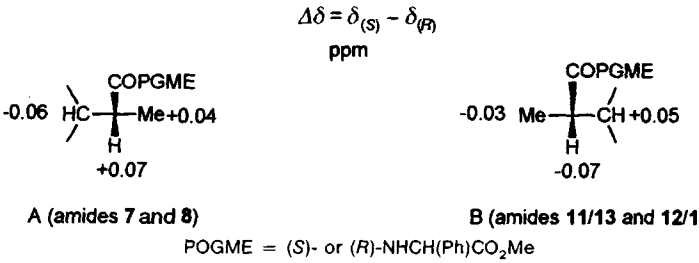
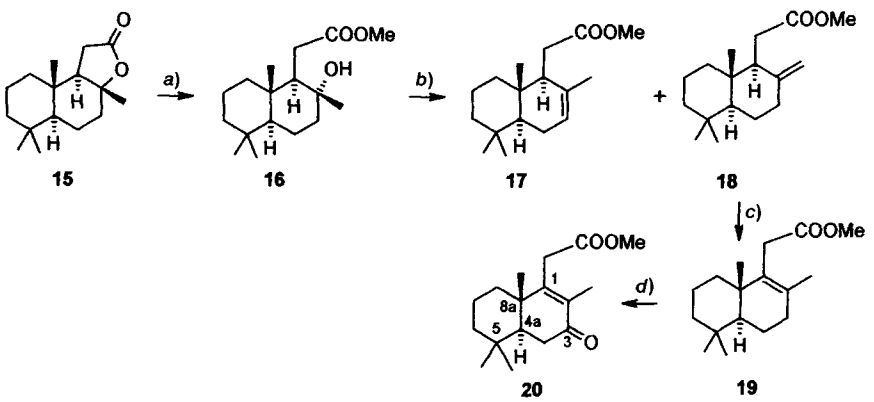


Figure. *Chemical-shift differences* $\Delta\delta$ [ppm] *of diastereoisomers* **7** *and* **8** (**A**), **11** *and* **12** (**B**), *and* **13** *and* **14** (**B**), *obtained from the mixture of the free carboxylic-acid precursors of esters* **3**, **6**, *and* **10** *with* (S)- *or* (R)-*phenyl glycine methyl ester.*

An unsuccessful attempt was then made at assigning the absolute configuration of **9** at the carbobicyclic moiety: poor yields in MTPA esterification of the equatorial allylic alcohol obtained from $NaBH_4$ reduction of the scarcely available **9** prevented the application of *Mosher*'s NMR methodology. Thus, recourse was made to empirical molar-rotation additivity rules [8] by calculating $[\varphi]_D = +90$ for both **3** and **5** as well as $[\varphi]_D = -226$ and $+222$ for relevant labdanes of the enantiomeric (levorotatory) and normal (dextrorotatory) series, respectively [9]. According to these calculations, compounds **9** and **10**, having $[\varphi]_D = 0.0$, should also belong to the *ent*-labdane series, in analogy with another epidioxy derivatives [6b]. In contrast, according to empirical rules correlating the sign of *Cotton* effects for *trans*-enones [10], compound **9**, showing three *Cotton* bands at λ_{max}(EtOH) 212, 254, and 339 nm of +, −, and + sign, respectively, should belong to the normal labdane series. In an attempt to resolve this discrepancy, model enone **20** which belongs to the normal labdane series was prepared from (+)-(3a*R*)-sclareolide (**15**) *via* **16**–**19** (*Scheme*). Of the three *Cotton* bands for **20** at λ_{max}(EtOH) 210, 241, and 340 nm, the crucial one at 210 nm indeed suggests the normal labdane configuration for **9** and **10**. The observation of the anomalous positive sign for the 237-nm band of **20** may be rationalized by a preferred conformation with the carbomethoxy group in the octant opposite to the angular Me group. This conformation

[1]) *Mosher*'s MTPA esters (= α-methoxy-α-(trifluoromethyl)benzene-acetates), obtained in very low yields from the products of catalytic (10% Pd/C) hydrogenation of either **3** or **5**, did not allow consistent assignments, and carboxylic-acid deprotection of **3** caused further problems of isolation. Thiourea and glutathione failed to reduce **3** selectively at the peroxide group.

Scheme



[a]) MeOH, *Amberlyst-15*, 48 h, r.t., then TLC; 54% of **16** and 46% of **15**. [b]) **16** and 2 equiv. of MsCl, dry pyridine, 12 h, then FC (SiO_2); **17**/**18** 1:2. [c]) CH_2Cl_2, *Amberlyst-15*, 12 h, r.t.; 100% of **19** (from **18**). [d]) Dimethyldioxirane, HPLC (SiO_2); 60%.

is indeed at the lowest potential-energy minimum according to molecular-mechanics calculations.

3. Bioassays. – The epidioxy esters **1**–**3**, **6**, and **10** were submitted to *in vitro* experiments against *Plasmodium falciparum*, the human parasite responsible for the most severe cases of malaria (*Table*). Deoxydiacarnoate B benzyl ester (**10**) proved the most active of the pure compounds, especially against a chloroquine (CQ)-resistant strain. Even more active was a 47:33:20 mixture of free carboxylic acids corresponding to the esters **3**, **6**, and **10**, respectively. This is in line with enhanced antimicrobial and cytotoxic activity (not reported) of these epidioxy acids as compared to their esters. However, this mixture of free acids has a poor security index (3.1(HeLa)/2.9), which was not evaluated for **10**. Methyl 3-epinuapapuanoate (**2**) [3] has a better security index (> 74/7.4) and,

Table. *Cytotoxicity against* Plasmodium falciparum *and Tumour Cell Lines* (IC_{50} M · 10^6)

	CQ-sensitive *P. falciparum*[a])		CQ-resistant *P. falciparum*[a])		Tumour cell lines		
	HB3	F32	FCR3	D2	KB	HeLa	Hep-2
1	7.1	0.01	21		> 70	> 70	> 70
2	7.4		7.2		> 74	> 74	> 74
3		124		73	2.1[b])		
6		73		62			
10		11		6.4			
Mixture[c])	2.9		1.4		2.5	3.1	2.1
CQ	0.03		0.23	0.21		> 0.1 [11]	

[a]) CQ = Chloroquine.
[b]) Experiment performed in Noumea (all other experiments done in La Paz).
[c]) 47:33:20 Mixture of free acids corresponding to **3**, **6**, and **10**, respectively.

interestingly, a similar activity on CQ-sensitive and CQ-resistant strains of *P. falciparum*. Combining this observation with the larger availability of natural ester **2** from the sponge, this compound was selected for *in vivo* experiments on *Plasmodium berghei*, a rodent malaria. Compound **2** was delivered to mice (*Exper. Part*) at 25 and 50 mg/kg showing, respectively, 56% growth inhibition with all mice surviving and 49% growth inhibition with one mouse dying and 4 surviving. At 100 mg/kg, compound **2** killed all the mice, probably by an anticoagulant process as the mice presented symptoms of hemorrhage.

Compounds **2** and **10**, like the mixture of free acids corresponding to esters **3**, **6**, and **10**, proved more effective on CQ-resistant strains than on CQ-sensitive strains of the parasite. This suggests that the antimalarial mode of action of these epidioxy-terpenes on parasite growth may be different from that of the quinolinamines. Earlier studies have evidenced the susceptibility of *Plasmodium*-infected erythrocytes to oxidant-mediated damages [12]. Survival of parasite and host cell depends on the delicate balance between oxidant stress and defence mechanisms. Artemisinin and its derivatives contain a peroxyde group which is involved in their antimalarial activity and, indeed, artemisinin induces lipid peroxidation *in vitro* [13]. On this basis, we may tentatively envision that the terpene peroxides examined here, though having the peroxide group in a very different arrangement than in artemisinin, exert their effects similarly to the latter in creating an oxidant stress on a similar model. This could also explain the side effects observed in mice. The above results stimulate assaying other epidioxy-terpenes among the many examples isolated from marine sponges and elaborating on them chemically, with the prospect to find a new lead in this new class of compounds active against CQ-resistant malaria parasites.

We thank the ORSTOM diving team for collecting the sponge, *J. Vacelet* for the taxonomy of the sponge, and, for skilled technical contributions, *S. Gadotti* (product isolation), *J. Callapa* and *G. Ruiz* (bioassays), and *A. Sterni* (mass spectra). Financial support from *MURST*, Roma (Progetti di Interesse Nazionale), *CNR*, Roma (including also the Progetto Strategico 96-05073), the *International Foundation for Sciences*, Sweden, and the *Fondo Nacional del Medio Ambiante*, Bolivia, is gratefully acknowledged. This work was carried out within the collaborative *ORSTOM-CNRS Program on Marine Substances of Biological Interest*.

Experimental Part

General. All evaporations were carried our at r.t. at reduced pressure. Yields are given on reacted reagents. TLC: *Merck* silica gel *60 PF_{254}*. Reversed-phase flash chromatography (FC): *Merck LiChroprep RP-18* (40–63 μm). HPLC: *Merck-LiChrosorb Si-60* (7 μm); for reversed-phase, *Merck LiChrosorb RP-18* (7 μm); in both cases 25 × 1 cm columns; solvent flow 5 ml min^{-1}; UV monitoring at 255 nm. UV: *Perkin-Elmer-Lambda-3* spectrophotometer (λ_{max} in nm, ε in mol^{-1} l cm^{-1}). Polarimetric data: *JASCO-DP-181* polarimeter, $[\alpha]_D$ values in 10^{-1} deg ml g^{-1}. CD: *JASCO-J-710* spectropolarimeter; λ [nm] ($\Delta\varepsilon$ [deg × l/mol × cm]). NMR: *Varian-XL-300*, ^{13}C at 75.43 and ^{1}H at 299.94 MHz; δ in ppm, in $CDCl_3$ rel. to internal $SiMe_4$ (= 0 ppm), probe temp. 20°; multiplicities and C and H assignments from DEPT, $^{1}H,^{1}H$-COSY, $^{1}H,^{13}C$-COSY, HMBC (= heteronuclear multiple bond coherence spectroscopy); terpene numbering. MS: *m/z* (rel. %); *Kratos MS80* with home-built acquisition system, electron ionization. Molecular-mechanics calculations were carried out with PCMODEL by *Serena Software*, which is based on the MMX force field.

Isolations. HPLC (hexane/AcOEt 49:1) of the mixture of benzyl esters obtained from the 731M/R1528 sample of *D. levii* [3] gave *ent*-muqubilin benzyl ester (**6**) (t_R 10 min; 16 mg, 0.0018% on freeze-dried sponge weight) and deoxydiacarnoate B benzyl ester (**10**) (t_R 13 min; 10 mg, 0.0011%). HPLC (hexane/AcOEt 4:1) of the mixture of methyl esters from the same source [3] gave diacarnoate B methyl ester (**9**) (t_R 10 min; 13 mg, 0.0014%).

Biological Assays. In vitro. Plasmodium falciparum CQ-sensitive strains (HB3 and F32) and CQ-resistant strains (FCR3 and D2) were kindly supplied by Prof. *Hagai Ginsburg*, Institute of Life Sciences, Jerusalem, and cultured according to *Trager* and *Jensen* [14] on glucose-enriched RPMI 1640 medium supplemented with HEPES and 10% human serum at 37°. The experiments, adapted from *Desjardin et al.* [15], were conducted in 96 flat-bottom well plates. Each well, containing 0.2 ml of a ring-stage culture (1% hematocrit, 2% parsitaemia), was exposed to increased concentrations of the test compounds. Control wells received the solvent and test compounds. After 24 h on culture conditions, [^{3}H]hypoxanthine (*Amersham*, London) was added (3 μCi/ml), and after an additional 24 h incubation period, cells were harvested in triplicate. Filters were dried for 2 h at 100° and counted in a toluene-based scintillation liquid. The percentage of growth inhibition of *P. falciparum* was calculated by the equation (DPM in control) – (DPM with test compound) × 100/(DPM in control), where DPM stands for the number of disintegrations per min.

In vivo. The *in vivo* antimalarial activity of methyl 3-epinuapapuanoate (**2**) [3] was determined in mice by the classical 4-days suppressive test [16] against *Plasmodium berghei*, a rodent malaria. Swiss male mice, mean body weight 20 ± 2 g, were infected with 107 parasitized cells in 0.9% saline medium, on day 0. Groups of 5 mice were treated intraperitoneally from day 0 to day 3 with increasing doses of compound **2** from 25 mg/kg up to 100 mg/kg. The suppressive effects were estimated on day 4, examining *Giemsa*-stained thin blood smears made from the tail of the treated mice and compared with a control group of mice treated with the soln. of the test compound. The stained thin blood smears were examined under × 1000 magnification, and the percentage of parasitized red blood cells was counted on at least 9000 red blood cells observed for each concentration. Percent growth inhibition of the parasite was calculated by the equation: (parasitaemia in control – parasitaemia with drugs) × 100 parasitaemia in control. In this mode, the reference drug (chloroquine) displayed an IC_{50} of 2.5 mg/kg.

ent-*Muqubilin Benzyl Ester* (= *(αR,3S,6R)-α,6-Dimethyl-6-[(E)-4-methyl-6-(2,6,6-trimethylcyclohex-1-en-1-yl)hex-3-onyl]-1,2-dioxane-3-acetic Acid Benzyl Ester*; **6**). $[\alpha]_D^{25} = -24.8$ ($c = 0.33$, $CHCl_3$). ^{1}H-NMR: 2.70 (br. *dq*, $J(2,3) = 7.0$, $J(2,24) = 7.0$, H–C(2)); 4.13 (*m*, H–C(3)); 1.66 (*m*, 2 H–C(4)); 1.64 (*m*, 2 H–C(5)); 1.50 (*m*, 2 H–C(7)); 2.01 (*m*, 2 H–C(8)); 5.10 (br. *t*, $J(9,8) = 7.1$, H–C(9)); 2.00 (*m*, 2 H–C(11)); 2.03 (*m*, 2 H–C(12)); 1.90 (br. *t*, $J(15,16) = 6.5$, 2 H–C(15)); 1.57 (*m*, 2 H–C(16)); 1.41 (*m*, 2 H–C(17)); 0.98 (*s*, Me(19), Me(20)); 1.59 (*s*, Me(21)); 1.63 (br. *s*, Me(22)); 1.27 (*s*, Me(23)); 1.28 (*d*, $J(24,2) = 7.0$, Me(24)); 5.14 (*s*, PhCH_2); 7.35 (*m*, Ph). ^{13}C-NMR: 173.58 (*s*, C(1)); 43.03 (*d*, C(2)); 81.39 (*d*, C(3)); 23.44 (*t*, C(4)); 31.89 (*t*, C(5)); 80.12 (*s*, C(6)); 39.62 (*t*, C(7)); 21.62 (*t*, C(8)); 123.22 (*d*, C(9)); 136.42 (*s*, C(10)); 40.18 (*t*, C(11)); 27.80 (*t*, C(12)); 137.06 (*s*, C(13)); 126.90 (*s*, C(14)); 32.73 (*t*, C(15)); 19.53 (*t*, C(16)); 39.81 (*t*, C(17)); 34.95 (*s*, C(18)); 28.59 (2*q*, C(19), C(20)); 19.80 (*q*, C(21)); 15.99 (*q*, C(22)); 20.68 (*q*, C(23)); 13.67 (*q*, C(24)); 66.40 (*t*, PhCH_2); 128.13 (2*d*), 128.23 (*d*), 128.53 (2*d*), 135.76 (*s*, Ph). MS: 482 (1, $M^{+\cdot}$), 319 (4), 181 (18), 137 (95), 91 (100).

Diacarnoate B Methyl Ester (= *(αS,3S,6R)-α,6-Dimethyl-6-{2-[(4aS,8aS)-3,4,4a,5,6,7,8,8a-octahydro-3-oxo-2,5,5,8a-tetramethylnaphthalen-1-yl]ethyl}-1,2-dioxan-3-acetic Acid Methyl Ester*; **9**). $[\alpha]_D^{25} = -2.8$ ($c = 0.32$, $CHCl_3$). CD (EtOH, 7.5×10^{-5} M): 212 (+5.2), 254 (−1.4), 339 (+0.7). ^{1}H-NMR: 2.61 (br. *dq*, $J(2,3) = 8$, $J(2,19) = 7.2$, H–C(2)); 4.24 (*m*, H–C(3)); 1.72 (*m*, 2 H–C(4)); 1.65 (*m*, 2 H–C(5)); 1.58 (*m*, 2 H–C(7)); 2.25 (*m*, 2 H–C(8)); 2.47 (*dd*, $J_{gem} = 17.5$, $J(12\beta,13) = 4.0$, H_β–C(12)); 2.33 (*dd*, $J_{gem} = 17.5$, $J(12\alpha,13) = 14.0$, H_α–C(12)); 1.66 (*dd*, $J(13,12\alpha) = 14.0$, $J(13,12\beta) = 4.0$, H–C(13)); 1.19 (*ddd*, $J_{gem} = 14.0$, $J(15\beta,16\alpha) = 13.0$, $J(15\beta,16\beta) = 4.0$, H_β–C(15)); 1.46 (*dddd*, $J_{gem} = 14.0$, $J(15\alpha,16\alpha) = J(15\alpha,16\beta) = 3.0$, $J(15\alpha,17\alpha) = 1.0$, H_α–C(15)); 1.68 (*m*, H_β–C(16)); 1.58 (*m*, H_α–C(16)); 1.89 (*dddd*, $J_{gem} = 13.0$, $J(17\alpha,16\alpha) = 4.0$, $J(17\alpha,16\beta) = 2.0$, $J(17\alpha,15\alpha) = 1.0$, H_α–C(17)); 1.31 (*td*, $J_{gem} = 13.0$, $J(17\beta,16\alpha) = 13.0$, $J(17\beta,16\beta) = 4.0$, H_β–C(17)); 1.15 (*d*, $J(19,2) = 7.2$, Me(19)); 1.35 (*s*, Me(20)); 1.74 (*s*, Me(21)); 1.06 (*s*, Me(22)); 0.86 (*s*, Me(23)); 0.90 (*s*, Me(24)); 3.70 (*s*, MeO). ^{13}C-NMR: 174.44 (*s*, C(1)); 42.62 (*d*, C(2)); 81.66 (*d*, C(3)); 22.63 (*t*, C(4)); 31.94 (*t*, C(5)); 79.90 (*s*, C(6)); 38.64 (*t*, C(7)); 22.77 (*t*, C(8)); 167.46 (*s*, C(9)); 130.31 (*s*, C(10)); 200.20 (*s*, C(11)); 35.20 (*t*, C(12)); 50.24 (*d*, C(13)); 33.11 (*s*, C(14)); 41.27 (*t*, C(15)); 18.59 (*t*, C(16)); 35.90 (*t*, C(17)); 41.08 (*s*, C(18)); 12.88 (*q*, C(19)); 20.37 (*q*, C(20)); 11.33 (*q*, C(21)); 18.12 (*q*, C(22)); 32.51 (*q*, C(23)); 21.32 (*q*, C(24)); 51.98 (*q*, MeO). EI-MS: 420 (3, $M^{+\cdot}$), 333 (4), 233 (16), 205 (100). HR-EI-MS: 420.287 ± 0.005 ($C_{25}H_{40}O_5^{+\cdot}$; calc. 420.287).

Deoxydiacarnoate B Benzyl Ester (= *(αS,3R,6R)-α,6-Dimethyl-6-{2-[(4aS,8aS)-3,4-4a,5,6,7,8,8a-octahydro-2,5,5,8a-tetramethylnaphthalen-1-yl]ethyl}-1,2-dioxan-3-acetic Acid Benzyl Ester*; **10**). $[\alpha]_D^{25} = 0.0$ ($c = 0.30$, $CHCl_3$). ^{1}H-NMR: 2.60 (br. *dq*, $J(2,3) = 8$, $J(2,19) = 7.0$, H–C(2)); 4.28 (*m*, H–C(3)); 1.67 (*m*, 2 H–C(4)); 1.67 (*m*, 2 H–C(5)); 1.50 (*m*, 2 H–C(7)); superimposed between 1.9–2.1 (*m*, 2 H–C(8)); superimposed between 1.9–2.1 (*m*, 2 H–C(11)); 1.48 (*m*, 2 H–C(12)); 1.10 (*dd*, $J(13,12\alpha) = 13.0$, $J(13,12\beta) = 2.0$, H–C(13)); superimposed at 1.4 (*m*, 2 H–C(15)); 1.58 (*m*, H_β–C(16)); 1.44 (*m*, H_α–C(16)); 1.78 (br. *d*, $J_{gem} = 14.0$, H_α–C(17)); 1.12 (*ddd*, $J_{gem} = 14.0$, $J(17\beta,16\alpha) = 13.0$, $J(17\beta,16\beta) = 4.0$, H_β–C(17)); 1.17 (*d*, $J(19,2) = 7.0$, Me(19)); 1.31 (*s*, Me(20)); 1.56 (*s*, Me(21)); 0.92 (*s*, Me(22)); 0.81 (*s*, Me(24)); 0.86 (*s*, Me(23)); 5.14 (*s*, PhCH_2); 7.35 (*m*, Ph). ^{13}C-NMR: 173.69 (*s*, C(1)); 42.86 (*d*, C(2)); 81.54 (*d*, C(3)); 22.66 (*t*, C(4)); 32.09 (*t*, C(5)); 80.31 (*s*, C(6)); 40.40

(*t*, C(7)); 21.04 (*t*, C(8)); 139.61 (*s*, C(9)); 126.16 (*s*, C(10)); 33.60 (*t*, C(11)); 19.03 (*t*, C(12)); 51.85 (*d*, C(13)); 33.30 (*s*, C(14)); 41.76 (*t*, C(15)); 19.03 (*t*, C(16)); 37.01 (*t*, C(17)); 39.10 (*s*, C(18)); 12.74 (*q*, C(19)); 20.09 (*q*, C(20)); 19.44 (*q*, C(21)); 20.09 (*q*, C(22)); 21.68 (*q*, C(23)); 33.30 (*q*, C(24)); 66.40 (*t*, PhCH); 128.13 (2*d*), 128.23 (*d*), 128.53 (2*d*), 135.76 (*s*, Ph). MS: 482 (1, $M^{+\cdot}$), 319 (11), 204 (14), 91 (100).

Conversion of **10** *to* **9**. Compound **10** (10 mg) was treated with an excess of freshly prepared (*via* peroxymonosulfate oxidation of acetone) dimethyldioxirane in acetone at 0° for 2 h, followed by prep. TLC (petroleum ether/Et_2O 1:1): **9** (R_f 0.6, 5 mg) and a mixture of epoxides (R_f 0.8, 5 mg).

Amides **7**, **8**, **11**/**13**, *and* **12**/**14**. A mixture (10 mg) of free carboxylic acids of **3**, **6**, and **10**, obtained by TLC (SiO_2, petroleum ether/Et_2O 1:1) [3], was treated with 1.2 mol-equiv. of dicarbonylbis[1*H*-imidazole] in dry pyridine (1.5 ml) under stirring for 45 min at r.t. Two mol-equiv. of (−)-(*R*)-phenylglycine methyl ester [7] were then added. The mixture was stirred overnight and then subjected to prep. TLC (AcOEt/AcOH 99:1) to give a mixture of amides (4 mg) and a mixture of unreacted free carboxylic acids (5 mg). HPLC (SiO_2, hexane/AcOEt 4:1) of the amides gave **8** (t_R 10 min, 1.0 mg) and **12**/**14** (t_R 11 min, 1.5 mg).

A similar experiment with (+)-(*S*)-phenylglycine methyl ester [7] gave 4 mg of amide mixture which was subjected to HPLC (*Si60*, hexane/AcOEt 3:1): **11**/**13** (t_R 10 min, 1.2 mg) and *7* (t_R 13 min, 1.0 mg).

*(*S*)-*N*-(ent-Muqubilin-1-yl)-2-phenylglycine Methyl Ester* (**7**): ^{1}H-NMR: 6.61 (N*H*/CHPh); 5.48 (NHC*H*Ph); 2.54 (H–C(2′)); 4.09 (H–C(3′)); 1.20 (Me(24′)).

*(*R*)-*N*-(ent-Muqubilin-1-yl)-2-phenylglycine Methyl Ester* (**8**): ^{1}H-NMR: 6.91 (N*H*CHPh); 5.55 (NHC*H*Ph); 2.47 (H–C(2′)); 4.15 (H–C(3′)); 1.16 (Me(24′)).

*(*S*)-*N*-(Deoxydiacarn-B-1-yl)-2-phenylglycine Methyl Ester* (**11**): ^{1}H-NMR: 6.90 (N*H*CHPh); 5.56 (NHC*H*Ph); 2.47 (H–C(2′)); 4.15 (H–C(3′)); 1.16 (Me(24′)).

*(*R*)-*N*-(Deoxydiacarn-B-1-yl)-2-phenylglycine Methyl Ester* (**12**): ^{1}H-NMR: 6.61 (N*H*CHPh); 5.48 (NHC*H*Ph); 2.54 (H–C(2′)); 4.10 (H–C(3′)); 1.19 (Me(24′)).

*(*S*)-*N*-(2-Epimuqubilin-1-yl)-2-phenylglycine Methyl Ester* (**13**): ^{1}H-NMR: 6.90 (N*H*CHPh); 5.56 (NHC*H*Ph); 2.47 (H–C(2′)); 4.15 (H–C(3′)); 1.16 (Me(24′)).

*(*R*)-*N*-(2-Epimuqubilin-1-yl)-2-phenylglycine Methyl Ester* (**14**): ^{1}H-NMR: 6.61 (N*H*CHPh); 5.48 (NHC*H*Ph); 2.54 (H–C(2′)); 4.10 (H–C(3′)); 1.19 (Me(24′)).

*(4a*S*,8a*S*)-3,4,4a,5,6,7,8,8a-Octahydro-3-oxo-2,5,5,8a-tetramethylnapththalene-1-acetic Acid Methyl Ester* (**20**). A soln. of (+)-(3a*R*)-sclareolide (*Aldrich*) (60 mg, 0.24 mmol) in MeOH (5 ml) was stirred in the presence of *Amberlyst-15* at r.t. for 48 h. The soln. was taken by syringe and submitted to prep. TLC (SiO_2 (2 mm thick), petroleum ether/Et_2O 1:1): *(1*R*,2*R*,4a*S*,8a*S*)-decahydro-2-hydroxy-2,5,5,8a-tetramethylnaphthalene-1-acetic acid methyl ester* (**16**; R_f 0.5; 54%) and recovered **15** (46%).

Labile **16** was used immediately by stirring it in dry pyridine with 2 equiv. of MsCl for 12 h. Then FC (SiO_2) gave a 1:2 mixture *(1*S*,4a*S*,8a*S*)-1,4,4a,5,6,7,8,8a-octahydro-2,5,5,8a-tetramethylnaphthalene-1-acetic acid methyl ester* (**17**)/*(1*S*,4a*S*,8a*S*)-decahydro-5,5,8a-trimethyl-2-methylenenaphthalene-1-acetic acid methyl ester* (**18**) in 65% yield.

The mixture **17**/**18** in CH_2Cl_2 was stirred in the presence of *Amberlyst-15* for 12 h at r.t. to give *(4a*S*,8a*S*)-3,4,4a,5,6,7,8,8a-octahydro-2,5,5,8a-tetramethylnaphthalene-1-acetic acid methyl ester* (**19**; 100% from **18**), while **17** did not react.

Compound **19** was treated with dimethyldioxirane in acetone overnight. Then the mixture was subjected to HPLC (SiO_2 hexane/AcOEt 85:15): **20** (t_R 8 min; 60%, 22% from **15**). CD (EtOH, 8.99×10^{-5} M): 210 (+4.9), 241 (+3.3), 340 (+0.9).

REFERENCES

[1] See Web Site http://www.malaria.org.

[2] B. Mons, E. Klasen, R. van Kessel, T. Nchinda, *Science* **1998**, *279*, 498.

[3] M. D'Ambrosio, A. Guerriero, C. Debitus, J. Waikedre, F. Pietra, *Tetrahedron Lett.* **1997**, *38*, 6285.

[4] L. V. Manes, G. J. Bakus, P. Crews, *Tetrahedron Lett.* **1984**, *25*, 931.

[5] R. J. Capon, J. K. MacLeod, *Tetrahedron* **1985**, *41*, 3391.

[6] a) J. Tanaka, T. Higa, K. Suwanborirux, U. Kokpol, G. Bernardinelli, C. W. Jefford, *J. Org. Chem.* **1993**, *58*, 2999; b) R. J. Capon, J. K. MacLeod, *J. Nat. Prod.* **1987**, *50*, 225; M. S. Butler, R. J. Capon, *Aust. J. Chem.* **1991**, *44*, 77.

[7] Y. Nagai, T. Kusumi, *Tetrahedron Lett.* **1995**, *36*, 1853.

[8] R. J. Capon, S. J. Rochfort, S. P. B. Ovenden, *J. Nat. Prod.* **1997**, *60*, 1261.

[9] S. Habtemariam, A. I. Gray, C. Lavaud, G. Massiot, B. W. Skelton, P. G. Watermann, A. H. White, *J. Chem. Soc., Perkin Trans. 1* **1991**, 893; H. Suzuki, M. Noma, N. Kawashima, *Phytochemistry* **1983**, *22*, 1294.
[10] J. K. Gawronski, *Tetrahedron* **1982**, *38*, 3; D. N. Kirk, *ibid.* **1986**, *42*, 777.
[11] A. Valentin, F. Benoit-Vical, C. Moulis, E. Stanislas, M. Mallie, I. Fouraste, J. M. Bastide, *Antimicrob. Agents Chemother.* **1997**, *41*, 2305.
[12] J. L. Vennerstrom, J. W. Eaton, *J. Med. Chem.* **1988**, *31*, 1269; A. O. Wozencraft, *Parasitology* **1986**, *92*, 559; E. Marva, J. Golenser, A. Cohen, N. Kitrossky, R. Har-el, M. Chevion, *Trop. Med. Parasitol.* **1992**, *43*, 17.
[13] M. D. Scott, S. R. Meshnick, R. A. Williams, D. T. Y. Chiu, H. C. Pan, *J. Labor. Clin. Med.* **1989**, 401.
[14] W. Trager, J. B. Jensen, *Science* **1976**, *193*, 673.
[15] R. Desjardin, C. Canfield, D. Haynes, J. Chulay, *Antimicrob. Agents Chemother.* **1979**, *16*, 710.
[16] W. Peters, 'Chemotherapy and Drug Resistance in Malaria' Academic Press, New York, 1970, pp. 64–136.

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