71. Verapliquinones: Novel Diprenylquinones from an *Aplidium* sp. (Ascidiacea) of Ile-Verte Waters, Brittany

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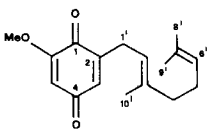
(23.I.87)

An *Aplidium* sp. (Ascidiacea), collected in French Atlantic waters near the Ile Verte, is shown to contain verapliquinone B (**2**) and verapliquinone D (**6**) as the first examples of linear diprenylquinones of the neryl type, besides their respective isomers **1** and **5** of the geranyl type.

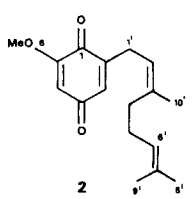
1. Introduction. – Many marine organisms contain prenylated quinones and hydroquinones. Limiting our interest to linear prenyl derivatives, which are analogues of the electron-transporting essential metabolites ubiquinones [1], the most common such sources are marine plants of the order Fucales. Many of them contain tetraprenyl- [2a, b], triprenyl- [2c], and diprenylquinones and/or hydroquinones [2d]. Less common sources are marine invertebrates such as sponges of the genus *Ircinia,* order Dictyoceratida (polyprenylquinones [3]), alcyonaceans [4a] and gorgonaceans (tetraprenylquinones [4b][1])), and ascidians of the order Aplousobranchia, family Polyclinidae. Members of the latter group contain monoprenylquinones, diprenylquinones, and a chromenol-type diprenylhydroquinone, as with *Aplidium californicum* [6a], *A. cavernosa* [6b], and *A. constellatum* [6c], respectively.

We report here on novel linear diprenylquinones from an *Aplidium* sp. The novelty lies in these compounds being neryl derivatives whilst all previously reported natural linear diprenylquinones are of the geranyl type [2d] [6b].

2. Results and Discussion. – The most abundant of the products described here, verapliquinone A (**1**), could only be isolated in sizable amounts by HPLC as a 4:1 mixture with verapliquinone B (**2**). Only under analytical HPLC conditions could the two compounds be separated, thus serving for UV spectra. However, NMR spectra in C_6D_6 of the above mixture allow us to assign the signals for each compound. This, together with mass spectra, solves the structural problem as follows.



1



2

[1]) A peculiar case is that of the marine *Sarcodictyon roseum* (Anthozoa, Stolonifera) which contains sarcodictyenone, a unique, optically active, ring-reduced derivative of a linear tetraprenylated quinone [5].

The presence of a quinone group in **1** is suggested by a strong UV absorption at 264 nm, IR absorptions at 1675 and 1650 cm^{-1}, and a typical ($M^{+\cdot}$ + 2) ion [7] in the MS. The ^{1}H-NMR spectrum *(Table 1)* suggests a 6-methoxy-substituted 1,4-benzoquinone bearing a hydrocarbon chain at C(2). This is indicated by the signals at 6.43 and 5.86 ppm for H–C(3) and H–C(5), which are mutually coupled by 2.8 Hz, and a typical *s* at 3.80 ppm for the MeO group. The ^{13}C-NMR spectrum *(Table 2)* confirms these conclusions showing 2 *d* at 132.85 and 107.17 ppm for C(3) and C(5) and a *q* at 56.27 ppm for MeO, besides 2 *s* at 182.2 and 187.7 ppm for the C=O groups. The presence, in the ^{13}C-NMR spectrum of **1**, of only 4 other olefinic resonances for two trisubstituted double bonds clearly suggests a linear diprenyl chain in agreement with the MS (*Exper. Part*). The fact that the chain bears 3 Me groups at double bonds implies the presence of a terminal isopropylidene group. The position of the other double bond as shown in structure **1** can be assigned on the basis of the double irradiation at 2H–C(1') (whose position is known by the deshielding effect of the quinone group) causing the *tq* of H–C(2') to lose a coupling of J = 7.8 Hz.

Structure **2** for verapliquinone B can be similarly derived from the NMR data in *Table 1*.

Table 1. *^{1}H-NMR Data for Verapliquinone A* (**1**), *B* (**2**), *C* (**5**), *and D* (**6**)

Proton(s) at[a])	**1** ($CDCl_3$)	**1** (C_6D_6)
C(3)	6.43 (*dt*, J(3,5) = 2.8, J(3,1') = 2.2)	6.36 (*dt*, J(3,5) = 2.4, J(3,1') = 2.2)
C(5)	5.86 (*d*, J(5,3) = 2.8)	5.42 (*d*, J(5,3) = 2.4)
C(1')	3.12 (br. *d*, J(1',2') = 7.8)	2.97 (br. *d*, J(1',2') = 7.3)
C(2')	5.13 (*tq*, J(2',1') = 7.6, J(2',10') = 1.2)	5.04 (*tq*, J(2',1') = 7.3, J(2',10') = 1.1)
C(4')	2.03 (*m*)	1.98 (br. *t*, J(4',5') = 7.4)
C(5')	2.03 (*m*)	2.07 (*q*, J(5',4') ≈ J(5',6') = 7.4)
C(6')	5.08 (br. *t*, J(6',5') = 7.2)	5.13 (br. *t*, J(6',5') = 7.4)
C(8')	1.67 (*d*, J(8',6') = 1.2)	1.74 (br. *d*, J(8',6') = 1.1)
C(9')	1.58 (br. *s*)	1.54 (br. *s*)
C(10')	1.60 (*d*, J(10',2') = 1.2)	1.42 (br. *d*, J(10',2') = 1.1)
MeO	3.80 (*s*)	2.80 (*s*)
OH	–	–
Proton(s) at[a])	**2** ($CDCl_3$)	**2** (C_6D_6)
C(3)	6.48 (*dt*, J(3,5) = 2.8, J(3,1') = 2.2)	6.40 (*dt*, J(3,5) = 2.4, J(3,1') = 2.2)
C(5)	5.86 (*d*, J(5,3) = 2.8)	5.44 (*d*, J(5,3) = 2.4)
C(1')	3.12 (br. *d*, J(1',2') = 7.8)	3.02 (br. *d*, J(1',2') = 7.3)
C(2')	5.13 (*tq*, J(2',1') = 7.6, J(2',10') = 1.2)	5.04 (*tq*, J(2',1') = 7.3, J(2',10') = 1.1)
C(4')	2.03 (*m*)	1.98 (br. *t*, J(4',5') = 7.4)
C(5')	2.07 (*m*)	2.07 (*q*, J(5',4') ≈ J(5',6') = 7.4)
C(6')	5.08 (br. *t*, J(6',5') = 7.2)	5.13 (br. *t*, J(6',5') = 7.4)
C(8')	1.64 (*d*, J(8',6') = 1.6)	1.65 (*d*, J(8',6') = 1.4)
C(9')	1.57 (br. *s*)	1.52 (br. *s*)
C(10')	1.74 (*q*, J(10',2') ≈ J(10',4') = 1.5)	1.59 (*q*, J(10',2') ≈ J(10',4') = 1.5)
OMe	3.80 (*s*)	2.80 (*s*)
OH	–	–
Proton(s) at[a])	**5** ($CDCl_3$)	**5** (C_6D_6)
C(3)	6.43 (*dt*, J(3,5) = 2.4, J(3,1') = 2.1)	6.38 (*dt*, J(3,5) = 2.4, J(3,1') = 2.1)
C(5)	5.86 (*d*, J(5,3) = 2.4)	5.41 (*d*, J(5,3) = 2.4)
C(1')	3.13 (br. *d*, J(1',2') = 7.6)	2.98 (br. *d*, J(1',2') = 7.3)
C(2')	5.13 (*tq*, J(2',1') = 7.6, J(2',10') = 1.2)	5.07 (*tq*, J(2',1') = 7.3, J(2',10') = 1.1)
C(4')	2.10 (br. *t*, J(4',5') = 7.2)	1.89 (*t*, J(4',5') = 7.4)
C(5')	1.28 (*m*)	1.24 (*m*)
C(6')	1.45 (*m*)	1.38 (*m*)
C(8')	1.21 (*s*)	1.05 (*s*)
C(9')	1.21 (*s*)	1.05 (*s*)
C(10')	1.62 (*d*, J(10',2') = 1.1)	1.44 (*d*, J(10',2') = 1.2)
OMe	3.81 (*s*)	2.80 (*s*)
OH	1.45 (br. *s*)	1.28 (br. *s*)

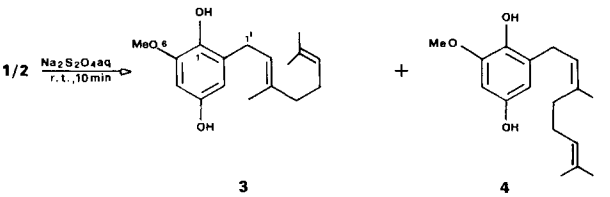
Table 1 (cont.)

Proton(s) at [a])	**6** ($CDCl_3$)	**6** (C_6D_6)
C(3)	6.45 (*dt*, *J*(3,5) = 2.4, *J*(3,1') = 2.1)	6.40 (*dt*, *J*(3,5) = 2.4, *J*(3,1') = 2.1)
C(5)	5.86 (*d*, *J*(5,3) = 2.4)	5.43 (*d*, *J*(5,3) = 2.4)
C(1')	3.13 (br. *d*, *J*(1',2') = 7.6)	3.00 (br. *d*, *J*(1',2') = 7.6)
C(2')	5.13 (*tq*, *J*(2',1') = 7.6, *J*(2',10') = 1.1)	5.07 (*tq*, *J*(2',1') = 7.3, *J*(2',10') = 1.1)
C(4')	2.10 (*t*, *J*(4',5') = 7.4)	1.91 (*t*, *J*(4',5') = 7.4)
C(5')	1.28 (*m*)	1.24 (*m*)
C(6')	1.45 (*m*)	1.38 (*m*)
C(8')	1.19 (*s*)	1.02 (*s*)
C(9')	1.19 (*s*)	1.02 (*s*)
C(10')	1.74 (*q*, *J*(10',2') = 1.5)	1.60 (*q*, *J*(10',2') = 1.5)
OMe	3.81 (*s*)	2.80 (*s*)
OH	1.45 (br. *s*)	1.28 (br. *s*)

[a]) The number of protons is defined by the appropriate structural formula.

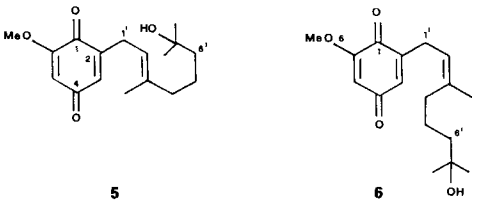
The configuration at the C(2'), C(3') double bond for **1** and **2** can be firmly established on the basis of the relatively low-field and high-field signal for C(10') of the (*Z*) compound **2** and the sterically more congested (*E*) compound **1**, respectively [8]. Structure **1** and **2** are confirmed by the transformation of their 4:1 mixture into a 4:1 mixture of the corresponding hydroquinones **3** and **4** *(Scheme 1)*.

Scheme 1



3 **4**

A 4:1 mixture of verapliquinone C (**5**) and verapliquinone D (**6**) has also been isolated in minor amounts from our *Aplidium* sp. Such a mixture, rather than the separate components, had to be studied for the same reasons discussed above for **1**/**2**. Thus, close structural analogies of **5** to **1** and of **6** to **2** are evident from the NMR data in *Tables 1* and *2*. However, both the MS, owing to the loss of a H_2O molecule from the molecular ion, and a strong absorption at 3450 cm^{-1} in the IR spectrum, suggest the presence of an OH group in **5** and **6**. Accordingly, both **5** and **6** lack ^{13}C-NMR resonances for unsaturated C-atoms of an isopropylidene group. Their place is taken by a deshielded *s* (70.93 ppm)



5 **6**

Table 2. *^{13}C-NMR Data for Verapliquinone A* (**1**), *B* (**2**), *C* (**5**), *and D* (**6**)

C-Atom	**1** ($CDCl_3$)	**1** (C_6D_6)	**2** ($CDCl_3$)	**2** (C_6D_6)	**5** ($CDCl_3$)	**5** (C_6D_6)	**6** ($CDCl_3$)[b])	**6** (C_6D_6)[b])
C(1)	182.20 (*s*)	187.10 (*s*)	182.20 (*s*)	187.10 (*s*)	187.72 (*s*)	[a])		
C(2)	146.47 (*s*)	146.18 (*s*)	146.47 (*s*)	146.18 (*s*)	[a])	145.26 (*s*)		
C(3)	132.85 (*d*)	132.71 (*d*)	132.85 (*d*)	132.71 (*d*)	132.88 (*d*)	132.71 (*d*)		
C(4)	187.70 (*s*)	187.10 (*s*)	187.70 (*s*)	187.10 (*s*)	187.72 (*s*)	[a])		
C(5)	107.17 (*d*)	107.11 (*d*)	107.17 (*d*)	107.11 (*d*)	107.16 (*d*)	107.10 (*d*)		
C(6)	158.90 (*s*)	158.91 (*s*)	158.90 (*s*)	158.91 (*s*)	158.95 (*s*)	158.90 (*s*)		
C(1′)	27.13 (*t*)	27.43 (*t*)	26.96 (*t*)	27.43 (*t*)	27.20 (*t*)	27.51 (*t*)		
C(2′)	117.75 (*d*)	118.88 (*d*)	118.46 (*d*)	119.70 (*d*)	117.92 (*d*)	118.84 (*d*)	118.50 (*d*)	
C(3′)	140.07 (*s*)	139.40 (*s*)	140.07 (*s*)	139.40 (*s*)	139.91 (*s*)	139.69 (*s*)		
C(4′)	39.63 (*t*)	39.89 (*t*)	31.87 (*t*)	32.05 (*t*)	39.99 (*t*)	40.23 (*t*)	32.07 (*t*)	32.24 (*t*)
C(5′)	26.43 (*t*)	26.73 (*t*)	26.34 (*t*)	26.64 (*t*)	22.51 (*t*)	22.67 (*t*)		
C(6′)	123.85 (*d*)	124.51 (*d*)	123.85 (*d*)	124.51 (*d*)	43.48 (*t*)	43.55 (*t*)		
C(7′)	131.83 (*s*)	131.60 (*s*)	131.83 (*s*)	131.60 (*s*)	70.93 (*s*)	69.98 (*s*)		
C(8′)	25.69 (*q*)	25.86 (*q*)	25.69 (*q*)	25.86 (*q*)	29.30 (*q*)	29.47 (*q*)		
C(9′)	17.71 (*q*)	17.72 (*q*)	16.10 (*q*)	15.89 (*q*)	29.30 (*q*)	29.47 (*q*)		
C(10′)	16.10 (*q*)	15.89 (*q*)	23.50 (*q*)	23.40 (*q*)	16.08 (*q*)	15.89 (*q*)	23.42 (*q*)	23.36 (*q*)
MeO	56.27 (*q*)	55.03 (*q*)	56.27 (*q*)	55.03 (*q*)	56.30 (*q*)	55.07 (*q*)		

[a]) Not detected.

[b]) Scarcity of material (0.38 mg of **6** and 1.12 mg of **5** in 0.5 ml of solvent) allowed for compound **6** only to detect the signals notably different from those of **5**.

Scheme 2

$$\mathbf{5/6} \xrightarrow[\text{r.t., 1 h}]{\text{POCl}_3\text{, pyridine}} \mathbf{1/2}$$

for C(7') and a *t* (43.48 ppm) for C(6'). The configurational assignment at the C(2'),C(3') double bond is based on similar evidences as for **1** and **2** above. The structural assignments for verapliquinone C (**5**) and D (**6**) are confirmed by their interconversion into **1** and **2** with preservation of the configuration *(Scheme 2)* [2]).

We thank Dr. *L. Cabioch* and Miss Dr. *F. Lafargue* for taxonomic determinations, the *Station Biologique* of Roscoff for laboratory facilities, and, for financial support, the *M.P.I.* (Progetti di Interesse Nazionale), the *C.N.R.* (Roma), and the *Provincia Autonoma di Trento, Sezione Agricoltura.*

Experimental Part

1. *General.* Flash chromatography: *Merck Kieselgel 60*, 20–50 μm. TLC: *Merck* Si_{F254} plates. HPLC: *Merck-LiChrosorb Si-60* (7 μm) and *Merck-LiChrosorb RP8* (7 μm, reverse phase); 25 × 1 cm columns, 5 ml/min solvent flux for prep. conditions; 25 × 0.4 cm columns, 1 ml/min solvent flux for anal. conditions; monitoring by UV at 254 nm. [α]: *JASCO-DP-181* polarimeter. UV (λ_{max} in nm, ε in mol^{-1} l cm^{-1}): *Perkin-Elmer Lambda-3.* IR ($\tilde{\nu}_{max}$ in cm^{-1}): *Pye-Unicam SP3-100.* NMR: *Varian-XL-300* (^{13}C-NMR at 75.43 MHz, ^{1}H-NMR at 300 MHz): δ (ppm) relative to internal Me_4Si (= 0 ppm) and *J* in Hz. EI-MS (*m/z* (%)): home-built spectrometer based on the *ELFS-4-162-8-Extranuclear* quadrupole [9].

2. *Isolations.* The ascidian was collected on September 23rd, 1984, at low tide between the Ile Verte and the Station Biologique of Roscoff as small colourless colonies mostly on *Rhodymenia palmata* (Rhodophyceae) but also on *Fucus serratus* (Phaeophyceae) and was preliminary identified by Dr. *L. Cabioch* as an *Aplidium* sp., probahly *A. pallidum*[3]). The animals were carefully detached from the plants by means of a thin spatula and were immediately stored in 95% EtOH. Samples of non colonized plants were separately stored in EtOH for comparison and proved not to contain any of the metabolites described here for the *Aplidium* sp. Waring blender homogenization of the animals, repeated extraction, filtration, and EtOH evaporation led to a residue which was extracted first with petroleum ether and then with AcOEt, leaving 16.7 g of dry-animal residue. The petroleum ether fraction was evaporated to give 0.7 g of an oily residue which was subjected to flash chromatography with hexane/Et_2O gradient elution. The mixture **1/2** was eluted close to, and largely contaminated by, sterols and fatty acids, whilst **5/6** was eluted in the next more polar fractions. Extensive reverse-phase HPLC with CH_3CN/H_2O 65:35, followed by HPLC with hexane/$(i\text{-}Pr)_2O$ 3:2 gave 0.005 g (0.03% of dry-animal weight) light-yellow **1/2** (4:1) and 0.0025 g (0.015%) light-yellow **5/6** (4:1). These mixtures could only be separated by HPLC under anal. conditions by collecting heads and tails only of the elution bands, *i.e.* discarding most of the materials as mixtures, using the same solvent mixtures as for the prep. conditions. All these compounds are pale yellow oils which tend to darken on exposure to air and light.

3. *Verapliquinone A/Verapliquinone B, 4:1 Mixture (= (2'E)/(2'Z)-2-(3',7'-Dimethylocta-2',6'-dienyl)-6-methoxy-1,4-benzoquinone;* **1/2**). Optically inactive. UV ($CHCl_3$): 342 (1370), 264 (13500; identical UV for separated, pure **1** or **2**). IR (neat): 1675*m*, 1650*s*, 1600*s*, 1230*s*. MS: 276 (21, $M^{+\cdot}$ + 2), 274 (2, $M^{+\cdot}$), 191 (50), 176 (5), 154 (24), 152 (19), 69 (93).

4. *Verapliquinone C/Verapliquinone D, 4:1 Mixture (= (2'E)/(2'Z)-2-(7'-Hydroxy-3',7'-dimethylocta-2'-enyl)-6-methoxy-1,4-benzoquinone;* **5/6**). Optically inactive. UV: 342 (1200), 265 (9800; identical UV for separated, pure **5** or **6**). IR (neat): 3450*vs*, 1680*m*, 1650*s*, 1600*s*, 1230*s*. MS: 292 (0.5, $M^{+\cdot}$), 277 (14, $M^{+\cdot}$ – Me), 276 (5, $M^{+\cdot}$ + 2), 274 (4, $M^{+\cdot}$), 191 (81), 154 (41), 152 (100), 69 (44).

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[2]) However, there is some evidence (see *Exper. Part*) of competition by the *Hoffmann* pathway.

[3]) However, samples of *Aplidium pallidum* collected in June 1984 in Iles des Glenans waters (French Atlantic) by scuba diving, and identified by Dr. *F. Lafargue*, do not contain any of the metabolites described here for the *Aplidium* sp. of the Ile Verte. We plan a taxonomic reexamination of the latter species in due time.

5. *Reduction of* **1/2** *to (2'E)/(2'Z)-2-(3',7'-Dimethylocta-2',6'-dienyl)-6-methoxy-1,4-hydroquinone* (**3/4**). To 1.5 mg (0.0055 mmol) of **1/2** (4:1) in 0.5 ml of Et_2O, a few drops of a soln. of 0.13 g of $Na_2S_2O_4$ in 1 ml of H_2O were added. The two-phase mixture was vigorously stirred for 10 min. Then, the Et_2O phase was evaporated to give 1.2 mg (80%) of pale yellow, liquid **3/4** (4:1 mixture) which tends to be oxidized in contact with air (→ **1/2** 4:1 mixture). MS: 276 (100, $M^{+\cdot}$), 191 (46), 175 (20), 154 (84), 152 (40), 69 (59).

Data for **3**: ^{1}H-NMR (C_6D_6): 6.13 (*d, J* = 2.8, H–C(5)); 6.00 (*d, J* = 2.8, H–C(3)); 5.57 (*tq, J* = 7.6, 1.1, H–C(2')); 5.22 (br. *t, J* = 7.4, H–C(6')); 5.18 (*s*, 2OH); 3.57 (*d, J* = 7.6, 2H–C(1')); 3.07 (*s*, CH_3O); 2.15 (*q, J* = 7.4, 2H–C(5')); 2.12 (*t, J* = 7.4, 2H–C(4')); 1.70 (*d, J* = 1.1, 3H–C(10')); 1.67 (br. *s*, 3H–C(9')); 1.52 (br. *s*, 3H–C(8')).

Data for **4**: ^{1}H-NMR (C_6D_6): 6.11 (*d, J* = 2.8, H–C(5)); 6.02 (*d, J* = 2.8, H–C(3)); 5.55 (br. *t, J* = 7.6, H–C(2')); 5.22 (br. *t, J* = 7.4, H–C(6')); 5.18 (*s*, 2OH); 3.59 (*d, J* = 7.6, 2H–C(1')); 3.07 (*s*, CH_3O); 2.25–2.15 (*m*, 2H–C(4'), 2H–C(5')); 1.74 (*q, J* = 1.6, 3H–C(10')); 1.67 (br. *s*, 3H–C(9')); 1.56 (br. *s*, 3H–C(8')).

6. *Dehydration of* **5/6**. To a soln. of **5/6** (4:1; 1 mg, 0.0034 mmol) in 1 ml of THF were added pyridine and $POCl_3$ in large excess. The mixture was stirred for 1 h. Then, a few ml of sat. aq. $CuSO_4$ soln. were added, and the mixture was extracted with Et_2O. The org. phase was evaporated to leave 0.8 mg containing essentially **1/2** (4:1), besides *ca.* 10% (HPLC) of 2 other compounds (4:1) which, on the basis of ^{1}H-NMR data alone (*m* at 4.78 for CH_2(8')), probably are the C(7'),C(8') double-bond isomers of **1/2**.

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