123. Bretonin A and Isobretonin A, Unique Glycerol Derivatives Isolated from a Demosponge of Brittany Waters

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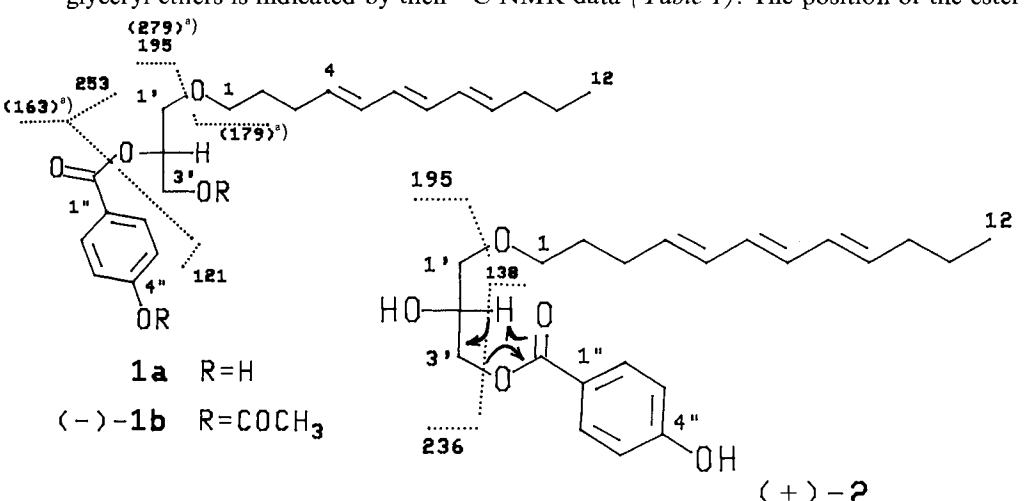
It is shown that an unidentified marine demosponge of Brittany contains two unique lipids consisting of glycerol etherified by a C_{12} trienic linear alcohol and esterified by 4-hydroxybenzoic acid. The latter is attached to the secondary position of glycerol in bretonin A (= 3-[((4*E*,6*E*,8*E*)-dodeca-4,6,8-trienyl)oxy]-2-(4-hydroxybenzoyl)propan-1-ol; **1a**), and to the other primary position of glycerol in isobretonin A (= (+)-3-[((4*E*,6*E*,8*E*)-dodeca-4,6,8-trienyl)oxy]-1-(4-hydroxybenzoyl)propan-2-ol; (+)-**2**). The structures are based on NMR and MS data, including the ones of the acetylation product (–)-**1b** of **1a**.

1. Introduction. – In connection with the discovery of long-chain acetylenic enol ethers of glycerol, called raspailynes, in marine sponges of the genus *Raspailia*, we have recently reviewed the literature on glycerol ethers [1]. In summary, with the exception of mutagenic polyolefinic glyceryl vinyl ethers, called fecapentaenes, isolated from human feces but of bacterial origin [2], glyceryl ethers are typically marine natural products, occurring in a variety of phyla [1].

We report here on two unique glycerols, etherified by a C_{12} trienic alcohol and esterified by 4-hydroxybenzoic acid, isolated from a demosponge of Brittany waters.

2. Results and Discussion. – That both bretonin A (**1a**) and isobretonin A ((+)-**2**) are glyceryl ethers is indicated by their ^{13}C-NMR data (*Table 1*). The position of the ester



a) *m/z* Values within parentheses refer to (–)-**1b**.

Table 1. *^{13}C-NMR Data of Bretonin A* (**1a**), *the Acetyl Derivative* (−)-**1b**, *and Isobretonin A* ((+)-**2**). In $CDCl_3$, unless otherwise stated.

C-Atom[a])	**1a** (CD_3OD)	**1a**	(−)-**1b**[b])	(+)-**2**
C(1)	70.45 (*t*)	71.17	68.89	71.50
C(2)	30.41 (*t*)	29.70	29.70	29.26
C(3)	30.24 (*t*)	29.13	29.15	29.18
C(4)	134.10 (*d*)	132.89	133.10	132.47
C(5)	131.93 (*d*)[c])	131.29[c])	131.20[c])	131.71[c])
C(6)	132.05 (*d*)[c])	131.13[c])	131.03[c])	131.08[c])
C(7)	132.41 (*d*)[c])	130.47[c])	130.52[c])	130.47[c])
C(8)	132.29 (*d*)[c])	130.47[c])	130.54[c])	130.47[c])
C(9)	134.93 (*d*)	134.63	134.54	134.05
C(10)	35.95 (*t*)	34.88	34.88	34.88
C(11)	23.65 (*t*)	22.49	22.50	22.50
C(12)	14.02 (*q*)	13.70	13.70	13.70
C(1′)	71.78 (*t*)	70.37	70.94	70.93
C(2′)	75.08 (*d*)	73.18	71.14	69.08
C(3′)	62.05 (*t*)	63.28	63.06	65.88
C(1″)	122.37 (*s*)	122.02	122.06	122.02
C(2″)	132.98 (*d*)	132.11	131.38	132.03
C(3″)	116.11 (*d*)	115.27	121.44	115.26
C(4″)	163.58 (*s*)	160.47	154.45	160.23
COO	167.72 (*s*)	166.11	165.05	166.23

[a]) Arbitrary numbering. For systematic names, see *Exper. Part.*

[b]) In addition: 170.74 (*s*, *C*OOC(3′)); 168.86 (*s*, *C*H_3COOC(4″)); 20.80 (*q*, *C*H_3COOC(3′)); 21.15 (*q*, *C*H_3COOC(4″)).

[c]) Within the same column, these assignments can be interchanged.

moiety is revealed by the ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*), the nature of the ester moiety confirmed by UV and by long-range HETCOR experiments [3], and the structure of the aliphatic trienyl moiety established by NMR, COSY, and HETCOR experiments.

In their ^{13}C-NMR spectra, both **1a** and (+)-**2** show 3*t* and 1*d* in the typical O-deshielded region for glyceryl ethers, such as batyl alcohol [1b]. In the case of **1a**, a *d* at 73.18 ppm is assigned to C(2′) owing to coupling with H–C(2′); it reveals that one of the glycerol OH groups is esterified. In fact, H–C(2′) has the typical chemical shift (5.20 ppm) of a proton at an esterified secondary glyceryl position [1a]. In the case of (+)-**2**, esterification at C(3′) is indicated by a *t* for C(3′) at 65.88 ppm; the 2 H–C(3′) constitute the *AB* part (4.36 ppm) of a *ABX* system, typical of diastereotopic protons at an esterified primary glyceryl position [1a].

The presence of a 4-hydroxybenzoate-ester moiety in **1a** and (+)-**2**, compatible with the UV shoulder at 250 nm, is established by the ^{1}H-NMR pattern for 4 symmetrically arranged aromatic protons. Moreover, the δ(C) and δ(H) values fit for COO and OH substitution. The assignment is further supported by long-range HETCOR experiments [3] which show the correlation from one side of COO with H–C(2″) and from the other side of C(1″) with H–C(3″). Mass spectra provide further structural support, showing for both **1a** and (+)-**2**, the fragment *m/z* 121 which is characteristic of the kelletinins [4].

Strong UV absorptions at 281, 270, and 261 nm of both **1a** and (+)-**2** suggest a conjugated triene moiety which, on the basis of ^{1}H-NMR decoupling and COSY experiments, must be placed at the center of a saturated C_{12} chain. The corresponding C-atoms can be assigned by HETCOR [3]. Regarding the configuration of the triene moiety, the ^{1}H-NMR signals of its 'external' protons H–C(4) and H–C(9) are sufficiently separated from one another to allow for their assignment on irradiation at the adjacent CH_2 groups. Large *J* values for coupling of H–C(4) with H–C(5) on one side and of H–C(9) with H–C(8) on the other side allow us to assign the (*E*) configuration to the 'external' double bonds of the triene moiety. This is confirmed by typical δ(C) values for C(3) and C(10). The problem is less straightforward for the central double bond as the resonances of the four 'inner' protons largely overlap, giving rise to a complex pattern at 6–6.1 ppm in CD_3OD which is not resolved on solvent

Table 2. *^{1}H-NMR Data of Bretonin A* (**1a**), *the Acetyl Derivative* (−)-**1b**, *and Isobretonin A* ((+)-**2**). In $CDCl_3$, unless otherwise stated.

H-Atom[a])	**1a** (CD_3OD)	**1a**	(−)-**1b**[b])	(+)-**2**
2 H–C(1)	3.50 (*m*)	3.49 (*m*)	3.45 (*m*)	3.49 (*m*)
2 H–C(2)	1.62 (*quint.*, J = 7.3)	1.65 (*quint.*, J = 7.3)	1.63 (*quint.*, J = 7.2)	1.66 (*quint.*, J = 7.0)
2 H–C(3)	2.08 (*q*, J = 7.3)	2.12 (*q*, J = 7.3)	2.12 (*q*, J = 7.2)	2.12 (*q*, J = 7.0)
H–C(4)	5.60 (*dt*, J = 14.1, 7.3)	5.60 (*dt*, J = 14.1, 7.3)	5.60 (*dt*, J = 14.2, 7.2)	5.60 (*dt*, J = 14.7, 7.0)
H–C(5)				
H–C(6)	5.98–6.06 (*m*)	5.98–6.06 (*m*)	5.98–6.06 (*m*)	5.98–6.06 (*m*)
H–C(7)				
H–C(8)				
H–C(9)	5.65 (*dt*, J = 14.8, 7.4)	5.65 (*dt*, J = 14.8, 7.2)	5.65 (*dt*, J = 14.3, 7.3)	5.65 (*dt*, J = 14.3, 7.2)
2 H–C(10)	2.06 (*q*, J = 7.3)	2.03 (*q*, J = 7.2)	2.05 (*q*, J = 7.3)	2.03 (*q*, J = 7.2)
2 H–C(11)	1.40 (*sext*, J = 7.3)	1.39 (*sext*, J = 7.2)	1.39 (*sext*, J = 7.3)	1.39 (*sext*, J = 7.2)
3 H–C(12)	0.91 (*t*, J = 7.3)	0.88 (*t*, J = 7.2)	0.88 (*t*, J = 7.3)	0.88 (*t*, J = 7.2)
2 H–C(1′)	3.68 (*d*, J = 5.0)	3.72 (*ABX*, J_{AB} = 10.5, J_{AX} = 4.5, J_{BX} = 5.1)	3.65 (*ABX*, J_{AB} = 10.4, $J_{AX} = J_{BX}$ = 4.8)	3.53 (*ABX*, J_{AB} = 9.8, J_{AX} = 4.2, J_{BX} = 6.1)
H–C(2′)	5.19 (*quint.*, J = 5.0)	5.20 (*quint.*, J = 5.0)	5.40 (*ddd*, J = 6.6, 5.1, 3.9)	4.10 (*m*)
2 H–C(3′)	3.78 (*d*, J = 5.0)	3.95 (*d*, J = 5.0)	4.37 (*ABX*, J_{AB} = 12.0, J_{AX} = 3.9, J_{BX} = 6.6)	4.36 (*ABX*, J_{AB} = 11.7, J_{AX} = 5.1, J_{BX} = 5.5)
H–C(2″)	7.93 (*d*, J = 8.8)	7.93 (*d*, J = 8.8)	8.07 (*d*, J = 9.0)	7.94 (*d*, J = 8.8)
H–C(3″)	6.83 (*d*, J = 8.8)	6.84 (*d*, J = 8.8)	7.17 (*d*, J = 9.0)	6.85 (*d*, J = 8.8)

[a]) Arbitrary numbering. For systematic names, see *Exper. Part.*
[b]) In addition: 2.04 (*s*, CH_3CO); 2.32 (*s*, CH_3CO).

change ($CDCl_3$ or CD_3CN). However, should the configuration be (*Z*) at C(6)=C(7), the resonances for both C(5) and C(8) would have been expected at higher field (*ca.* 125 ppm [5]).

Isobretonin A ((+)-**2**) differs from bretonin A (**1a**) with regard to both the ^{1}H- and the ^{13}C-NMR signals of the glyceryl portion. In support of structure (+)-**2** are the O-deshielded ^{13}C-NMR signals; only the one at 69.08 ppm is a *d*, which allows us to assign it to C(2′); this C-atom is coupled to the proton appearing as a *m* at 4.10 ppm. The upfield shift of 1 ppm for H–C(2′) of (+)-**2** as compared to the signal of H–C(2′) of **1a** confirms the free alcoholic function at C(2′) of (+)-**2**. The MS fully confirms the structural assignment for (+)-**2**, showing the loss of the benzoate group and of H–C(2′) *via McLafferty* rearrangement [6].

Bretonin A (**1a**) undergoes acetylation, under standard conditions, at both the alcoholic and the phenolic function, giving (−)-**1b**. The MS of **1b** confirms the above structural assignments, showing the molecular ion and a conclusive fragmentation pattern (see *Formula* (−)-**1b**).

Among the few known natural esters/ethers of glycerol, typical are those of the gorgonian *Plexaurella dichotoma* [7a] and of the brown seaweed *Sargassum fulvellum* [7b]. In such cases, however, the esterifying/etherifying moieties consist of ordinary acids and alcohols: C_{16} [7a] or C_{20} fatty acids [7b] and saturated long-chain (C_{16}–C_{18}) alcohols [7a] or the residue of methacrylic acid [7b]. In contrast, bretonin A (**1a**) and isobretonin A ((+)-**2**) possess unusual ester and ether moieties. As regards the ester moieties, the closest formal examples are the kelletinins and the buccinulins, erythrityl tetrabenzoates isolated from the prosobranch mollusks *Kelletia kelletii* of the Caribbean [4] and *Buccinulum corneum* of the Mediterranean [8], respectively. As in the case of the kelletinins [4], the origin of the 4-hydroxybenzoic-acid moiety of **1a** and (+)-**2**, is expected as a member of the shikimate pathway, to have vegetable or microbial origin.

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Experimental Part

1. *General.* All evaporations were carried out at reduced pressure at r.t. TLC: *Merck Kieselgel 60 PF_{254}*. Flash chromatography (FC): *Merck Kieselgel Si60*, 15–25 μm. HPLC: *Merck LiChrosorb Si60* (7 μm). Reverse-phase HPLC: *Merck-LiChrosorb RP-18* (7 μm; 25 × 1 cm column). UV spectra: *Perkin-Elmer-Lambda-34* spectrophotometer; λ_{max} in nm, ε in $dm^3 \cdot mol^{-1} \cdot cm^{-1}$. Polarimetric data: *JASCO-DIP-181* digital polarimeter. ^{1}H-NMR and ^{13}C-NMR spectra: *Varian XL300* (300 or 75.43 MHz, resp.); δ (ppm) relative to internal Me_4Si (= 0 ppm) and J in Hz; ^{13}C multiplicities, APT [9] or DEPT [10] technique. EI-MS: home-built quadrupole mass spectrometer based on the *ELFS-4-162-8-Extranuclear* quadrupole [11].

2. *Collection and Isolations.* The sponge was collected by deep dredging off the North-Eastern coast of France in summer 1982. The fresh sponge was mechanically freed of H_2O, immersed in EtOH for some days, homogenized by a *Waring* blender, and filtered to give 63 g of residue (dry wt.). The filtrate was evaporated. The residue from the evaporation was extracted 3 times first with petroleum ether and then with AcOEt. Evaporation of the extracts led to 1.0 g and 0.7 g of oily residues, respectively, which were stored at −20°. Most unfortunately, the dry residue of the sponge was thrown away, and when the extracts have been resumed in October 1988 for study, the voucher specimen was no more found.

The AcOEt extract was subjected to FC with hexane/Et_2O gradient elution collecting 15 fractions of 100 ml each. *Fr. 8* and *9* were evaporated and the residues (33 and 42 mg) combined and subjected to reverse-phase HPLC (CH_3CN/H_2O 65:35, 5 ml/min) to give nearly pure **1a** (t_R 15.8 min) and (+)-**2** (t_R 17.1 min). Further purification by reverse-phase chromatography (CH_3CN/H_2O 75:25, 6 ml/min) gave pure **1a** (t_R 14.0 min, 4 mg) and (+)-**2** (t_R 14.9 min, 1.8 mg).

3. *3-[((4E,6E,8E)-Dodeca-4,6,8-trienyl)oxy]-2-(4-hydroxybenzoyl)propan-1-ol* (= *1-[((4E,6E,8E)-Dodeca-4,6,8-trienyl)oxy]-3-hydroxypropan-2-yl 4-Hydroxybenzoate;* **1a**). [α]: at $c = 0.10$ in $CHCl_3$, the α values from 589 to 435 nm were too small to be taken with confidence. UV ($CHCl_3$): 281 (21000), 270 (30000), 261 (27500), 250 (sh). MS: 253 (2), 195 (8), 121 (100).

4. *Acetylation of* **1a**. For 1 h, **1a** (3 mg) was stirred with excess Ac_2O/pyridine at r.t. Excess pyridine was exctracted with aq. $CuSO_4$ soln., AcOEt was added to the org. phase which was filtered on reverse-phase filters (*Whatman*). The raw product was subjected to HPLC with hexane/AcOEt 3:1, t_R 7 min, to give 1.9 mg of pure *(−)-3-[((4E,6E,8E)-dodeca-4,6,8-trienyl)oxy]-2-(4-acetoxybenzoyl)prop-1-yl acetate* (= *(−)-1-acetoxy-3-[((4E,6E,8E)-dodeca-4,6,8-trienyl)oxy]propan-2-yl 4-acetoxybenzoate;* (−)-**1b**). $[\alpha]_D^{25} = -7.0$, $[\alpha]_{546}^{25} = -8.5$, $[\alpha]_{435}^{25} = -17.7$, $[\alpha]_{365}^{25} = -30.8$ ($c = 0.13$, $CHCl_3$). UV ($CHCl_3$): 281 (26000), 271 (31000), 261 (26500), 241 (30200). MS: 458 (3, M^+), 279 (22, $[M - 179]^+$), 237 (25, $[279 - COCH_2]^+$), 179 (3, $[M - 279]^+$), 163 (33), 121 (100, $[163 - COCH_2]^+$).

5. *(+)-3-[((4E,6E,8E)-Dodeca-4,6,8-trienyl)oxy]-1-(4-hydroxybenzoyl)propan-2-ol* (= *(+)-3-[((4E,6E,8E)-Dodeca-4,6,8-trienyl)oxy]-2-hydroxypropyl 4-Hydroxybenzoate;* (+)-**2**). $[\alpha]_D^{25} = +7.3$, $[\alpha]_{546}^{25} = +10.0$, $[\alpha]_{435}^{25} = +16.4$, $[\alpha]_{365}^{25} = +31.8$ ($c = 0.11$, $CHCl_3$). UV ($CHCl_3$): 281 (22000), 271 (31000), 261 (28000), 250 (sh). MS: 236 (2), 195 (5), 138 (23), 121 (100).

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