Polar Metabolites of the Tropical Green Seaweed *Caulerpa taxifolia* Which Is Spreading in the Mediterranean Sea: Glycoglycerolipids and Stable Enols (α-Keto Esters)

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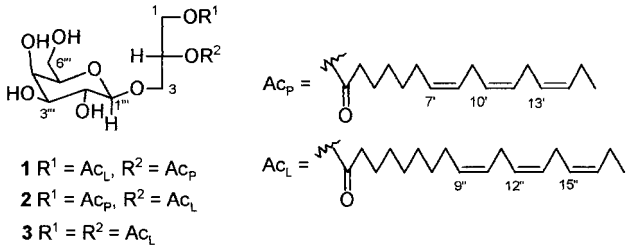
Examination of the polar components of the green seaweed *Caulerpa taxifolia* (VAHL) C. AGARDH, which is heavily spreading in the northeastern Mediterranean, led to two families of compounds. The new (2*R*)-3-*O*-β-D-galactopyranosyl-1-*O*-[(7*Z*,10*Z*,13*Z*)-hexadeca-7,10,13-trienoyl]-2-*O*-[(9*Z*,12*Z*,15*Z*)-octadeca-1,12,15-trienoyl]-*sn*-glycerol (**2**) was isolated in low abundance, like the analogues **1** and **3** already known from freshwater cyanobacteria. The acyl positions in **1**–**3** were determined by enzymatic methods and the absolute configuration from the *O*-galactosylglycerol obtained upon alcaline methanolysis. More abundant were the (4-hydroxyphenyl)- and (3,4-dihydroxyphenyl)pyruvic acid methyl esters, occurring in the enol (*Z*) forms **13a** and **14a** accompanied by very minor (*E*) forms **13b** and **14b**. The latter became predominant on UV irradiation of **13a** or **14a**, allowing the determination of the C=C configuration of these isolatable, stable enols from ^{1}H,^{13}C NMR couplings (larger H–C(3)/C(1) coupling constant in the (*E*) than in the (*Z*) isomer). Contrary to literature implications, the *O*-galactosylglycerolipids **1**–**3** lack any cholinergic or histaminergic activity; similarly, enols (= α-keto esters) **13** and **14** or terpenoids of this seaweed were also devoid of such biological activities (see *Table*).

1. Introduction. – Spreading of alien species is being observed at increased frequency [1] side-to-side to man-driven mass extinction [2]. Both phenomena threaten the biological diversity at any level, either of population [2a], species, or higher taxon [3]: mass extinction because extant species disappear faster than new species arise, and invasions because they prevent isolation which constitutes the basis for generating biological diversity [4].

A case in point is the current spreading of the tropical green seaweed *Caulerpa taxifolia* (VAHL) C. AGARDH (Ulvophyta, Caulerpales, Caulerpaceae) in the Mediterranean Sea. This is an unusual invasion since this seaweed has developed toxic, lipophilic terpenoids against herbivorous grazers to sustain its sparse and inconspicuous populations in the pressure for food of the tropics [5]. Following the colonization of the Mediterranean Sea, the content of toxic terpenoids of *C. taxifolia* has greatly increased [6], as well as the size of the fronds, the density of the populations, and the homeostatic capacities [5]. This incurs risks of decrease of the biological diversity and of ecotoxicity in the Mediterranean, unless the *in vitro* observed photodegradation of the algal terpenoids [5b] also occurs in nature. The prospects that this strengthened strain of *C. taxifolia* may find the way back to the tropics – in a form of natal homing [5c] – is most preoccupying.

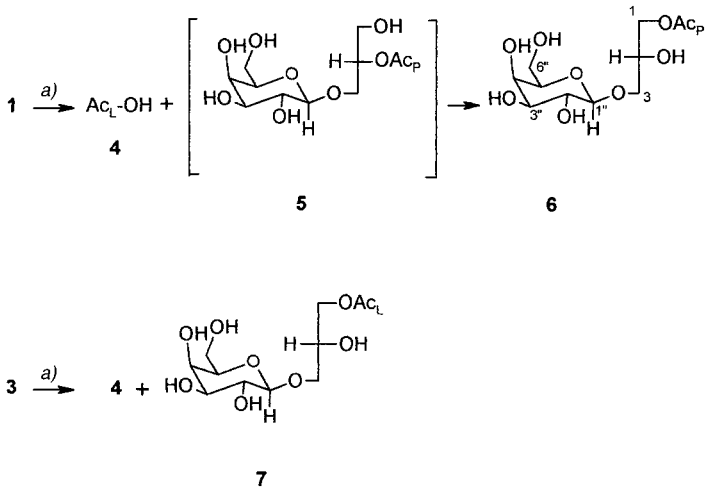
Aimed at a broader understanding of the metabolism of this seaweed, we have now studied the polar fractions of a Mediterranean strain of *C. taxifolia*, finding H_2O-soluble, stable enols and polar glycoglycerolipids which were assayed in the rat-isolated uterus and aorta.

2. Results and Discussion. – 2.1. *Glycoglycerolipids.* FAB-MS of medium-polarity products **1**–**3** from *C. taxifolia* gave the same composition, $C_{43}H_{70}O_{10}$, for **1** and **2**, and $C_{45}H_{74}O_{10}$ for **3**. The NMR data of **1**–**3** suggested the presence of a di-*O*-acylglycerol *O*-linked to a β-galactopyranose moiety and of six *cis* disubstituted C=C bonds for all these compounds. The nature and positions of the two polyunsaturated acyl groups were established by a combination of enzymatic and chemical hydrolysis (*Scheme 1*). Using a commercial lipase of type XI from *Rhizopus arrhizus*, which had already been used both in the structural analysis of mono-*O*-galactosylglycerols from marine cyanobacteria [7] and green algae [8], as well as in the synthesis of antifungal phospholipids [9], we obtained from **1** the mono-*O*-acylglycerol derivative **6** with a (7*Z*,10*Z*,13*Z*)-hexadeca-7,10,13-trienoyl (Ac_p) chain at the primary OH group, besides linolenic acid (**4**; *Scheme 1*); it should be noticed that no reaction occurred under these conditions in the absence of the enzyme. Compound **3** gave the *O*-galactosylglycerol derivative **7** and linolenic acid (Ac_L–OH; **4**). That enzymatic hydrolysis occurred at the primary-alcohol function of **1** to give the elusive intermediate **5**, followed by Ac_p migration, found also evidence in the slow methanolysis at the secondary-alcohol function on storage of **2** in CD_3OD to give **6** and ester **8** (*Scheme 2*).



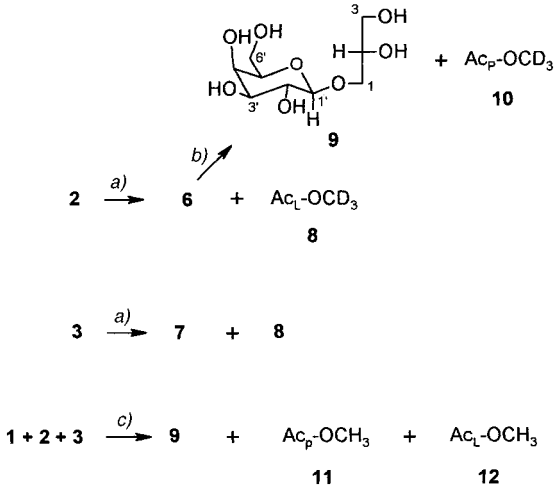
Contrasting reports have appeared for processes employing the above enzymes. Thus, selective lipase-XI-induced hydrolysis at the primary-alcohol function was first reported not only for **1** [7a] but also for other mono-*O*-galactosyldi-*O*-acylglycerols [7b] and di-*O*-acylated sulfoglycolipids and galactolipids [7c], all isolated from freshwater cyanobacteria [7a]. Subsequently, these processes were redescribed as being accompanied by an *sn*-2 $\rightarrow$ *sn*-1 acyl transposition of the type shown in *Scheme 1*, whose extent was judged to depend on the amount of lipase, type of buffer, presence or not of surfactants [7d,e], and reaction time [7d][10]. On the other hand, a complete transposition of the type shown in *Scheme 1* by lipase XI in similar systems required a higher pH (8.5) [9]. Our results are not in line with these reports since we observed complete *sn*-2 $\rightarrow$ *sn*-1 acyl transposition with the above and similar glycoglycerolipids *a*) at lower pH (7.7) than indicated in the synthetic procedure [9], *b*) in a boric acid/

Scheme 1



a) Lipase type XI (from *Rhizopus arrhizus*), *Triton X-100*, boric acid/borax buffer pH 7.7, 38°, 40 min.

Scheme 2



a) CD_3OD, 4° for 45 days (25% conversion), then 50° for 24 h (50% conversion). *b*) Addition of solid $NaHCO_3$, then HPLC. *c*) Sat Na_2CO_3/MeOH, r.t., 3 h.

borax buffer which was expected to give instead a mixture of the transposed and the non-transposed *O*-galactosylmono-*O*-acylglycerolipid [7d], *c*) in a shorter time than that indicated to give a mixture of transposed and non-transposed products [7d], and *d*) using a similar amount of lipase (900 units) that brought about instead regioselective 1-

deacylation [7c]. The possibility that the batch of lipase used in our work contained also an acyltransferase was not investigated, being outside the scope of our work[1]).

Glycoglycerolipids occur widely and copiously in vascular plants [13], raphidophycean microalgae [11], certain seaweeds [12], cyanobacteria [7], and marine dinoflagellates [14]. Compounds **1** and **3** were already known [7a,b,d][8][12b][15a–c], while **2** is a new isolate. As to the glycoglycerolipids of the green seaweeds (Ulvophyta), it is only known that the main *O*-galactosylglycerolipid of *Monostroma nitidum* (Ulotrichales) [12b] has a C16:4 fatty acid residue at C(2), in sharp contrast with products **1**–**3** from *C. taxifolia*.

The problem of the absolute configuration of **1**–**3** was then addressed by subjecting the mixture **1**–**3** to rapid methanolysis in the presence of Na_2CO_3 which gave the *O*-galactosylglycerol **9** and the methyl esters **11** and **12** (*Scheme 2*)[2]). Compound **9** was determined to be (2*R*)-1-*O*-β-D-galactopyranosyl-*sn*-glycerol by comparing the specific rotation and NMR spectra with published data [7a–c][13][15a][16]. This also established that the configuration of **2** is the same as for the *O*-galactosylglycerols of terrestrial plants [11]. This additional piece of evidence as to the relatedness of green seaweeds to vascular plants does not imply necessarily a similarity of functions of the metabolites, however. *O*-Galactosylglycerolipids **1**–**3**, being present in *C. taxifolia* in trace amounts, are unlikely to play a major role in stabilizing the algal membranes, in contrast to vascular plants. Since glycerolipids are known to play various other biological functions, the elucidation of the role they play in *C. taxifolia* remains a challenge.

2.2. *Enols (α-Keto Esters)*. Aryl pyruvic esters are more abundant in *C. taxifolia* than the glycerolipids. Both UV spectra (λ_{max} 299 nm, typical of the enol form of α-keto acids) and NMR spectra suggested that these compounds exist as mixtures of enol tautomers [17], one stereoisomer (**13a** or **14a**) being largely predominant in both cases. The stereoisomers proved stable and isolatable by HPLC. UV Irradiation (350 nm) of the predominant stereoisomer produced a mixture of stereoisomers in the ratio 45:55 in favour of the minor natural stereoisomer (**13b** or **14b**)[3]). By acetylation of the major isomeric enol forms **13a** or **14a**, the diacetyl and triacetyl derivatives **15** and **16**, respectively, were obtained (*Scheme 3*).

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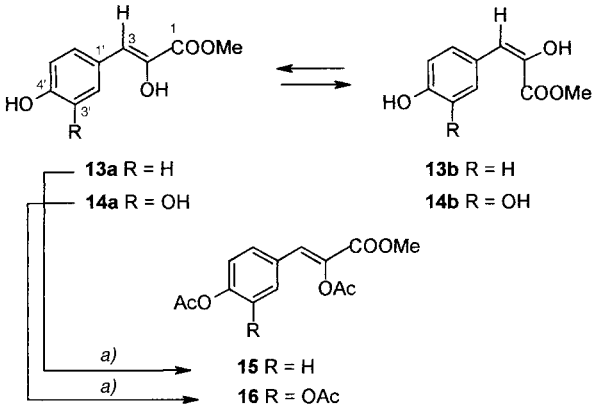
[1]) The course of the 1-*O*-deacylation of similar *O*-galactosylglycerolipids of raphidophycean microalgae by a bacterial type-XIII lipase was smoother, not accompanied by acyl transfer [11]. The enzymatic method proved also in this case an indispensable tool in assigning the position of the acyl chains of glycoglycerolipids, whilst chemical methods gave ambiguous results [11]. This casts doubt on the assignment by chemical or spectroscopic methods alone of the position of the acyl chains in similar glycoglycerolipids of brown [12a] and other seaweeds [12b].

[2]) Since the resulting *O*-galactosylglycerol **9** was diastereoisomerically pure (NMR), the use of the mixture **1**–**3** was to this purpose equivalent to that of the pure *O*-galactosyldi-*O*-acylglycerols. On storage of **2** in CD_3OD in a NMR probe, a partial conversion to **6** and **8** occurred (*Scheme 2, a*, and *Exper. Part*), and on addition of Na_2CO_3, **6** was converted to **9** and **10**. Similarly, on storage of **3** in CD_3OD, a partial conversion to **7** and **8** occurred. It is understood that in these experiments with **2** and **3** ^{1}H-NMR observation could not reveal D exchange at OH, whilst the isolated products had proton restored at OH following aqueous

[3]) This ratio did not change either on overnight standing of the irradiated mixture in the dark or on further UV irradiation (350 nm) for 1 h. By irradiation under identical conditions of a 1:2 mixture **13b**/**14b**, the same isomer ratio (45:55) for **13a**/**13b** and **14a**/**14b** was obtained. In contrast, similar irradiation of commercial (4-hydroxyphenyl)pyruvic acid resulted in only a 3% increase of the relative population of the minor isomeric enol form. The major isomer **13b** or **14b** revealed $^3J(H\text{–}C(3),C(1)) = 4.2$ Hz, suggesting (*Z*)-configuration (*vide infra*).

Scheme 3



a) Ac_2O, Py, r.t., 1 h.

The criterion of a larger $^3J(H,C_{trans})$ than $^3J(H,C_{cis})$ heterocoupling constant in assigning the C=C configuration of trisubstituted alkenes [18a,b] was recently applied to marine indole alkaloids, the aplysinopsins [18c], and was also useful in the case of the present enol forms. Thus, following the enrichment in **14b** of the natural mixture **14a**/**14b** by UV irradiation (350 nm), the stable stereoisomers **14a** and **14b** could be separated in pure forms by HPLC and their fully coupled ^{13}C-NMR spectra be recorded under selective heteronuclear decoupling of the Me resonances[4]). It could thus be determined that the major natural isomer **14a** has a small (3.7 Hz) H–C(3)/C(1) ^{1}H,^{13}C heterocoupling, indicating (*Z*)-configuration [18]. Correspondingly, the most abundant isomer from photoequilibration, **14b**, showed a larger heterocoupling (9.2 Hz), indicating (*E*)-configuration.

A number of different arylpropane-derived compounds have been previously isolated from *Caulerpa racemosa* from the eastern coasts of India [19]. Together with the enol forms **13a** and **14a** of α-keto ester, they further support the close phylogenetic relationship of the Caulerpales to vascular plants.

2.3. *Biological Assays.* Neither **1** nor the mixture **1**–**3** of natural composition had any significant effect on preparations of rat-isolated uterus under resting tension (*Table*, *a*). In the same myometrial strips, a maximally effective concentration of acetylcholine (ACh) (10^{-3} M) [20] elicited an initial, rapid phasic contraction (83.51 ± 6.83, expressed, here and below, in mg per mg of wet tissue) followed by rhythmic contractions. The ACh-induced contraction was completely abolished in the presence of atropine (10^{-6} M), demonstrating that the *O*-galactosyldi-*O*-acylglycerolipids assayed here are devoid of cholinergic activity. We also analysed the relaxant effects of *C. taxifolia* products in uterine strips pre-contracted by KCl or oxytocin. KCl-Induced contraction depends primarily on Ca^{2+} influx *via* L-type, voltage-operated Ca^{2+} channels [21], whereas the oxytocin-induced contraction involves additional mechanisms such as activation of receptor-operated Ca^{2+} channels, Ca^{2+} release from

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4) Otherwise, coupling of C(1) with the Me group led to such an extensive fragmentation of the signals that some of them became hardly distinguishable from background noise.

intracellular stores, and Ca^{2+}-independent processes [21][22]. In the uterus, isoosmotic addition of KCl (60 mM) caused a phasic contraction (70.15 ± 3.25) which then declined and was followed by a prolonged sustained plateau (40.80 ± 5.68). Oxytocin (0.2 nM) caused rhythmic contractions of stable intensity (99.73 ± 10.90) and duration. Compound **1** and the mixture **1**–**3** also failed to relax contractions evoked by either KCl (*Table*, *b*) or oxytocin (*Table*, *c*), suggesting that they do not interact with any of the above mentioned mechanisms. Compound **1** was also evaluated in the rat aorta, a preparation in which histamine induced an H1-mediated contraction [23], but again with negative results.

Table 1. *Functional Assays on Rat-Isolated Myometrium*[a]) *with Products Isolated from* Caulerpa taxifolia

Conc. [M]	**1**	**1**/**2**/**3** 5 : 2 : 3	**13a**/**14a** 1 : 2[b])	Caulerpenyne	Caulerpenyne/oxytoxin 1 2 : 1	Control
a) *Effect on preparations under resting tension*[c]):						
10^{-9}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.68 ± 0.01	0.00 ± 0.00
10^{-8}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.01 ± 0.01	0.00 ± 0.00
10^{-7}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.22 ± 0.01	0.00 ± 0.00
10^{-6}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.49 ± 0.02	0.00 ± 0.00
10^{-5}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.78 ± 0.02	0.00 ± 0.00
b) *Effect on preparations precontracted by a depolarizing solution* (60 mM KCl)[d]):						
10^{-9}	13.17 ± 0.03	0.00 ± 0.00	4.17 ± 0.04	1.54 ± 0.05	5.17 ± 0.02	4.76 ± 0.02
10^{-8}	23.70 ± 0.05	2.07 ± 0.02	6.25 ± 0.63	4.62 ± 0.03	11.99 ± 0.03	14.46 ± 0.07
10^{-7}	30.29 ± 0.07	9.69 ± 0.01	7.50 ± 0.08	6.15 ± 0.03	22.27 ± 0.06	16.99 ± 0.08
10^{-6}	32.91 ± 0.07	14.53 ± 0.02	11.66 ± 0.06	15.38 ± 0.07	27.43 ± 0.08	18.68 ± 0.09
10^{-5}	32.91 ± 0.07	22.47 ± 0.01	18.73 ± 0.07	35.38 ± 0.09	58.78 ± 0.10	23.17 ± 0.09
c) *Effect on preparations precontracted by oxytocin* (0.2 nM)[d]):						
10^{-9}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.43 ± 0.01	0.36 ± 0.01
10^{-8}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	31.88 ± 0.30	0.73 ± 0.01
10^{-7}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	38.57 ± 0.26	1.45 ± 0.01
10^{-6}	0.00 ± 0.00	0.36 ± 0.01	1.16 ± 0.01	0.00 ± 0.00	42.50 ± 0.24	2.46 ± 0.02
10^{-5}	0.00 ± 0.00	0.72 ± 0.01	2.33 ± 0.02	36.63 ± 0.32	103.44 ± 0.02	3.84 ± 0.03

[a]) Values are the mean ± s.e.m. in 5 different animals. [b]) Containing a small amount of **13b**/**14b** (see text). [c]) Data expressed as a percentage of the maximal contraction evoked by acetylcholine (1 nM). [d]) Data expressed as a percentage of the maximal contraction evoked by either KCl or oxytoxin, respectively, prior to the addition of the test product.

The above findings may have a bearing on work concerning an allegedly isolated product from Mediterranean *C. taxifolia* which was deemed to possess an 'aminoacidic skeleton in an alicyclic chain and polyenic conjugated bonds' and to have cholinergic and/or histaminergic activity [24]. Actually, the COSY plot reported [24] does not support any such structural conjecture but, although plotted at a too high threshold value to allow discerning all details, it is compatible with structures **1**–**3**. These cannot be responsible for the reported [24] biological activity, however, as demonstrated above, and therefore, we have considered possible contaminants of the isolated sample [24]. The enol forms **13a**/**13b** and **14a**/**14b** of α-keto esters were such candidates, of higher polarity than the *O*-galactosylglycerolipids, but they proved inactive in the rat myometrium assay (*Table*). In the same vein, we further conceded that, following

inappropriate separation procedures, the isolated sample [24] might have been contaminated with products of far lower polarity than the enol forms of the α-keto acids, like the algal sesquiterpenoids. Of these, the most abundant, caulerpenyne [25a] is known to display various biological activities, such as cytotoxicity towards both marine ciliates [25a] and human tumour cells [26][27], antibacterial activity [25a], as well as alteration of ionic signals involved in cell dynamics by influencing the calcium uptake [28]. Thus, both caulerpenyne and the related oxytoxin 1[5]) were examined in the rat-isolated-uterus assay. Oxytoxin 1 (assayed in a 1:2 mixture with caulerpenyne) showed non-negligible activity by both abolishing rhythmic contractions induced by oxytocin 0.2 nM and partially relaxing the KCl-induced response, whereas caulerpenyne showed only marginal inhibition of the KCl- and oxytocin-induced contractions. Since all these effects were only observed at the highest concentration assayed (10^{-5} M; see *Table*, *b* and *c*), neither oxytoxin 1 nor caulerpenyne can be responsible for the cholinergic and/or histaminergic activities reported [24].

One might wonder whether the summer sample of *C. taxifolia* from Imperia, used in previous work [24], might have contained other metabolites than our summer sample of the seaweed from Elba Island. Our examination of various strains of *C. taxifolia* – including tropical ones – by very sensitive and specific MS-MS tandem ionspray techniques [6] makes this possibility very unlikely. In any event, such a possibility is not suggested by the reported spectral data [24].

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Experimental Part

General. Lipase XI: from *Rhizopus arrhizus* (*Sigma*). All evaporations were carried out at r.t. under reduced pressure. Flash chromatography (FC): *Merck Si-60* (15–25 μm). TLC: *Merck* silica gel *60 PF_{254}* and *Merck RP-18 F_{254}*. Reversed-phase HPLC: *Merck LiChrospher RP18* (7 μm), 25 × 1 cm columns; UV monitoring at λ 220 nm and solvent flux 5 ml min^{-1}, if not otherwise specified. Polarimetric data: *JASCO-DP-181* polarimeter; $[\alpha]_D$ values in 10^{-1} deg ml g^{-1}. UV: *Perkin-Elmer-Lambda-3* spectrophotometer. NMR: *Varian-XL-300*, ^1H at 299.94 MHz and ^{13}C at 75.43 MHz; δ in ppm and *J* in Hz, in either CD_3OD or $CDCl_3$, rel. to the solvent residual signals, δ(H) 3.31 and δ(C) 49.00 ppm for the first and δ(H) 7.25 and δ(C) 77.00 ppm for the latter, where the solvent residual signals are rel. to $SiMe_4$ (= 0 ppm); assignments from DEPT, COSY60, selective decoupling irradiations, and ^1H,^{13}C COSY. MS (EI and FAB): *Kratos-MS80* with home-built computerized data-acquisition system and equipped with a *Vacumetrics-DIP* gun for FAB spectra.

Collection and Isolations. Caulerpa taxifolia was collected (856M) in the Baia di Calenzana, Elba Island, at the end of July 1997 and was immediately cleaned from epiphytes, immersed into 95% EtOH and stored in the cold. After a few days, a portion of the EtOH soln., corresponding to the extract from *ca*. 4 kg (wet weight) of seaweed, was homogenized and filtered, and the EtOH extract was evaporated to give 17.8 g of residue that was subjected to FC (gradient elution with hexane/AcOEt and AcOEt/MeOH and then pure MeOH) for a total of 34 fractions of 400 ml each. *Frs. 7–10* contained pure caulerpenyne and *Fr. 11* caulerpenyne/oxytoxin 1 2:1, which were used for the bioassays. *Fr. 26* (300 mg (dry weight)) was subjected to reversed-phase FC (MeOH/H_2O gradient), collecting 8 fractions of 60 ml each; of these *a*) *Fr. 6* (70 mg (dry weight)) was subjected to prep. TLC (AcOEt/MeOH 9:1) to give mixture **1**–**3**; the latter was subjected to reversed-phase HPLC (MeOH/H_2O 97:3) yielding pure **1** (t_R 9.5 min; 17.6 mg), **2** (t_R 10.5 min; 6.6 mg), and **3** (t_R 11.3 min; 9.2 mg); *b*) *Fr. 1* (130 mg

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[5]) Oxytoxin 1 was isolated from *C. taxifolia* [25a], but it might be an artifact of the hydrolysis of caulerpenyne during workup [25b] and thus might well have been a contaminant of the mixture used in previous work [24].

(dry weight)) was subjected to reversed-phase HPLC (MeOH/H_2O 1:9, monitoring at λ 254 nm) yielding **13b**/**14b** 1:2 (t_R 2.9 min; 3.8 mg) and pure **14a** (t_R 7.5 min; 80.0 mg) and pure **13a** (t_R 9.8 min; 39.5 mg).

Biological Assays. Preparation of uterine strips was done as previously described [20] with slight modifications as follows. Uterine horns from virgin female Wistar rats (200–250 g) in the oestrous stage of the hormonal cycle were removed and carefully cleaned. Longitudinal strips of uterine smooth muscle (8–10 mm in length and 1–2 mm in diameter) were prepared and mounted in tissue baths containing 4 ml of *Krebs* soln. of the following composition (mM): NaCl (118), KCl (5.6), $CaCl_2$ (1.9), $MgSO_4$ (0.95), $NaHPO_4$ (1.0), $NaHCO_3$ (25), and glucose (11). Preparations were suspended under an initial tension of 0.5 g, gassed with O_2/CO_2 95:5 and maintained at 37°. Mechanical responses were recorded isometrically by means of force-displacement transducers (*Grass FT-03*) connected to a *LETICA* amplifier and an *ABB-GOERZ-SE-130* multichannel recorder. At the beginning of the experiment and after a 45-min equilibration period, the preparation was induced to contract two or more times by administration of a maximally effective concentration of ACh (10^{-3} M) at 30 min intervals, until constant responses were obtained. The last response served as an internal standard for all experiments. Uterine strips were allowed to equilibrate for a further 60-min period before addition of cumulative concentrations of a *C. taxifolia* product (10^{-9}–10^{-5} M) or its vehicle (time-matched paired control strips). In a separate set of experiments, we examined the effect of the *C. taxifolia* products on uterine contractions evoked by a high K^+ depolarizing soln. (60 mM K^+, prepared by equimolar substitution of KCl for NaCl in the physiological soln.) or by the receptor agonist oxytoxin (0.2 nM). When the tonic contraction induced by KCl was sustained, or when the rhythmic contractions produced by oxytocin had attained a constant amplitude, a test product (1 nM – 10 μM) or its vehicle (time-matched control tissues) were added to the bath in a cumulative manner at 15-min intervals. Some experiments were carried out in isolated rings form rat aorta, essentially as described for the uterus, except for KCl replacing ACh as the spasmogen used as an internal standard. In all experiments, only one product was tested on each tissue. Values are expressed as mean ± s.e.m. for 5 experiments in 5 different animals. Contractile responses were measured as peak increases in force and expressed in mg per mg tissue (wet weight) or as a percentage of the peak increase in force produced by ACh (10^{-3} M). Relaxation responses were expressed as percentage inhibition of the maximum tension induced by the spasmogen (KCl or oxytocin) prior to the addition of the products.

*(2*R*)-3*-O-*β*-D-*Galactopyranosyl-2*-O-*[(7*Z*,10*Z*,13*Z*)-hexadeca-7,10,13-trienoyl]-1*-O-*[(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoyl]*-sn-*glycerol* (**1**). Oil. $[\alpha]_D^{25} = -2.7$ ($c = 0.2$, $CHCl_3$) [8]: $[\alpha]_D = -2.8$ ($c = 0.1$, $CHCl_3$). NMR: data matched those in [8]. FAB-MS (glycerol): 769 ($[M+Na]^+$).

*(2*R*)-3*-O-*β*-D-*Galactopyranosyl-1*-O-*[(7*Z*,10*Z*,13*Z*)-hexadeca-7,10,13-trienoyl]-2*-O-*[(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoyl]*-sn-*glycerol* (**2**). Oil. $[\alpha]_D^{25} = -2.8$ ($c = 0.2$, $CHCl_3$). ^{1}H-NMR (CD_3OD): 4.22 (*dd*, *J*(1a,2) = 6.5, J_{gem} = 12.1, H_a–C(1)); 4.44 (*dd*, *J*(1b,2) = 3.0, J_{gem} = 12.1, H_b–C(1)); 5.27 (*m*, H–C(2)); 3.74 (*dd*, *J*(3a,2) = 5.7, J_{gem} = 11.0, H_a–C(3)); 3.98 (*dd*, *J*(3b,2) = 5.4, J_{gem} = 11.0, H_b–C(3)); 2.31 (*t*, 2 H–C(2′)); 1.62 (*m*, 2 H–C(3′)); 1.38 (*m*, 2 H–C(4′), 2 H–C(5′)); 2.05 (*m*, 2 H–C(6′)); 5.30 (*m*, H–C(7′), H–C(8′), H–C(10′), H–C(11′), H–C(13′), H–C(14′)); 2.82 (*m*, 2 H–C(9′), 2 H–C(12′)); 2.09 (*m*, 2 H–C(15′)); 0.97 (*q*, *J*(16′,15′) = 7.5, Me(16′)); 2.33 (*t*, *J*(2″,3″) = 7.0, 2 H–C(2″)); 1.62 (*m*, 2 H–C(3″)); 1.38 (*m*, 2 H–C(4″) to 2 H–C(7″)); 2.09 (*m*, 2 H–C(8″)); 5.36 (*m*, H–C(9″), H–C(10″), H–C(12″), H–C(13″), H–C(15″), H–C(16″)); 2.82 (*m*, 2 H–C(11″), 2 H–C(14″)); 2.09 (*m*, 2 H–C(17″)); 0.97 (*q*, *J*(18″,17″) = 7.5, Me(18″)); 4.23 (*d*, *J*(1‴,2‴) = 7.2, H–C(1‴)); 3.52 (*dd*, *J*(2‴,1‴) = 7.2, *J*(2‴,3‴) = 9.6, H–C(2‴)); 3.45 (*dd*, *J*(3‴,2‴) = 9.6, *J*(3‴,4‴) = 3.3, H–C(3‴)); 3.83 (*dd*, *J*(4‴,3‴) = 3.3, *J*(4‴,5‴) = 1.0, H–C(4‴)); 3.51 (*ddd*, *J*(5‴,4‴) = 1.0, *J*(5‴,6‴a) = 5.4, *J*(5‴,6‴b) = 6.9, H–C(5‴)); 3.71 (*dd*, *J*(6‴a,5‴) = 5.4, J_{gem} = 11.3, H_a–C(6‴)); 3.77 (*dd*, *J*(6‴b,5‴) = 6.9, J_{gem} = 11.3, H_b–C(6‴)). ^{13}C-NMR (CD_3OD): 63.99 (*t*, C(1)); 71.81 (*d*, C(2)); 68.72 (*t*, C(3)); 175.03, 174.67 (2*s*, C(1′), C(1″)); 132.79, 132.73, 130.92, 130.86, 130.76, 129.27 (× 2), 129.16, 129.07 (× 2), 128.98, 128.23 (all *d*, C(7′), C(8′), C(10′), C(11′), C(13′), C(14′), C(9″), C(10″), C(12″), C(13″), C(5″), C(16″)); 35.07, 34.95, 30.85, 30.82, 30.72, 30.44, 30.34, 30.20, 30.18, 30.08, 29.81, 28.18, 28.14, 28.06, 26.55, 26.43, 26.03, 25.91 (all *t*, C(2′) to C(6′), C(9′), C(12′), C(15′), C(2″) to C(8″), C(11″), C(14″), C(17″)); 14.69 (*q*, C(16′), C(18″)); 105.36 (*d*, C(1‴)); 72.38 (*d*, C(2‴)); 74.87 (*d*, C(3‴)); 70.22 (*d*, C(4‴)); 76.80 (*d*, C(5‴)); 62.46 (*t*, C(6‴)). FAB-MS (glycerol): 769 ($[M+Na]^+$).

*(2*R*)-3*-O-*β*-D-*Galactopyranosyl-1,2-di*-O-*[(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoyl]*-sn-*glycerol* (**3**). Oil. $[\alpha]_D^{25} = -3.8$ ($c = 0.3$, $CHCl_3$) [15a]: $[\alpha]_D = -4.0$ ($c = 0.3$, $CHCl_3$). NMR: data matched those in [8][15a]. FAB-MS (glycerol): 797 ($[M+Na]^+$).

Enzymatic Hydrolysis of Compounds **1** *and* **3**. A soln. of **1** (4.0 mg) and *Rhizopus arrhizus* lipase XI (900 units) in the presence of *Triton X-100* (2.5 mg) in a boric acid/borax buffer (0.6 ml, pH 7.7) was stirred at 38° for 1 h. Then were added in sequence AcOH (0.1 ml) and EtOH (1.0 ml). The mixture was evaporated and the residue subjected to reversed-phase HPLC (MeOH/H_2O 85:15) to give *3*-O-*β*-D-*galactopyranosyl-1*-O-

[(7Z,10Z,13Z)-hexadeca-7,10,13-trienoyl]-sn-*glycerol* (**6**, t_R 5.9 min; 1.9 mg, 70%). MeOH eluates from column washing after each HPLC cycle were combined and evaporated and the residue was subjected to reversed-phase HPLC (MeOH/H_2O 95:5) to give *(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid* (= linolenic acid; **4**; t_R 8.5 min; 1.0 mg).

On the same treatment, **3** (3.2 mg) gave *3*-O-*β*-D-*galactopyranosyl-1*-O-*[(9Z,12Z,15Z)-octadeca-9,12,15-trienoyl]*-sn-*glycerol* (**7**; t_R 6.8 min; 1.3 mg) and **4** (0.8 mg).

Data of **4**: ^{1}H-NMR (CD_3OD): 2.33 (*t*, *J* = 7.0, 2 H–C(2)); 1.63 (*m*, 2 H–C(3)); 1.37 (*m*, 2 H–C(4) to 2 H–C(7)); 2.09 (*m*, 2 H–C(8), 2 H–C(17)); 5.36 (*m*, H–C(9), H–C(10), H–C(12), H–C(13), H–C(15), H–C(16)); 2.81 (*m*, 2 H–C(11), 2 H–C(14)); 0.98 (*q*, *J* = 7.5, Me).

Data of **6**: ^{1}H-NMR (CD_3OD): 4.15 (*m*, 2 H–C(1)); 3.99 (*m*, H–C(2)); 3.94, 3.68 (2*m*, 2 H–C(3)); 2.31 (*t*, *J* = 7.3, 2 H–C(2′)); 1.63 (*m*, 2 H–C(3′)); 1.38 (*m*, 2 H–C(4′), 2 H–C(5′)); 2.08 (*m*, 2 H–C(6′), 2 H–C(15′)); 5.35 (*m*, H–C(7′), H–C(8′), H–C(10′), H–C(11′), H–C(13′), H–C(14′)); 2.81 (*t*, *J* = 5.7, 2 H–C(9′), 2 H–C(12′)); 0.98 (*q*, *J* = 7.5, Me); 4.23 (*d*, *J* = 7.4, H–C(1″)); 3.51 (*m*, H–C(2″), H–C(5″)); 3.46 (*dd*, *J* = 9.6, 3.3, H–C(3″)); 3.81 (*dd*, *J* = 3.3, 1.0, H–C(4″)); 3.74 (*m*, 2 H–C(6″)). ^{13}C-NMR (CD_3OD): 175.36 (*s*); 132.70 (*d*); 130.84 (*d*); 129.20 (*d*); 129.13 (*d*); 128.98 (*d*); 128.18 (*d*); 105.29 (*d*); 76.74 (*d*); 74.81 (*d*); 72.53 (*d*); 71.85 (*d*); 70.24 (*d*); 69.59 (*t*); 66.55 (*t*); 62.44 (*t*); 34.87 (*t*); 30.37 (*t*); 29.79 (*t*); 27.99 (*t*); 26.48 (*t*); 26.37 (*t*); 25.84 (*t*); 21.40 (*t*); 14.62 (*q*). FAB-MS (glycerol): 509 ($[M+Na]^+$).

Data of **7**: ^{1}H-NMR (CD_3OD): 4.15 (*m*, 2 H–C(1)); 3.99 (*m*, H–C(2)); 3.94, 3.67 (2*m*, 2 H–C(3)); 2.33 (*t*, *J* = 7.0, 2 H–C(2′)); 1.63 (*m*, 2 H–C(3′)); 1.37 (*m*, 2 H–C(4′) to 2 H–C(7′)); 2.09 (*m*, 2 H–C(8′), 2 H–C(17′)); 5.36 (*m*, H–C(9′), H–C(10′), H–C(12′), H–C(13′), H–C(15′), H–C(16′)); 2.82 (*m*, 2 H–C(11′), 2 H–C(14′)); 0.98 (*q*, *J* = 7.5, Me); 4.22 (*d*, *J* = 7.4, H–C(1″)); 3.51 (*m*, H–C(2″), H–C(5″)); 3.46 (*dd*, *J* = 9.6, 3.3, H–C(3″)); 3.82 (*dd*, *J* = 3.3, 1.0, H–C(4″)); 3.74 (*m*, 2 H–C(6″)). FAB-MS (glycerol): 537 ($[M+Na]^+$).

Alkaline Hydrolysis of Compounds **1**–**3**. Compound **2** (3.3 mg), on storage in CD_3OD (0.6 ml) for 45 days at 4° and then 24 h at 50°, was partially converted into compounds **6** (see *Scheme 2, a*, and *Footnote 2*) and *(D$_3$)methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate* (**8**); NMR observation was carried out on products **6** and **8** after aqueous workup. On addition of catal., solid $NaHCO_3$, the hydrolysis went to completion, giving *1*-O-*β*-D-*galactopyranosyl*-sn-*glycerol* (**9**) and *(D$_3$)methyl (7Z,10Z,13Z)-hexadeca-7,10,13-trienoate* (**10**). The mixture was subjected to reversed-phase HPLC (MeOH/H_2O 95:5): **9** (t_R 2.5 min; 1.1 mg, 93%), **8** (t_R 9.8 min; 1.2 mg), and **10** (t_R 6.9 min; 1.1 mg).

Under similar conditions, $8 \cdot 10^{-3}$ M **3** in CD_3OD was partially converted into **7** and **8** (see *Footnote 2*).

In another experiment, sat. methanolic Na_2CO_3 soln. (1 ml) was added to **1**–**3** (3:1:2; 10.0 mg), stirring the mixture at r.t. until complete conversion (1 h, TLC). The residue from evaporation was subjected to reversed-phase HPLC (MeOH/H_2O 95:5): **9** (t_R 2.5 min; 3.2 mg, 90%), *methyl (7Z,10Z,13Z)-hexadeca-7,10,13-trienoate* (**11**; t_R 6.2 min; 3.0 mg), and *methyl (9Z,12 Z,15Z)-octadeca-9,12,15-trienoate* (**12**; t_R 8.9 min; 3.3 mg).

Data of **8**: ^{1}H-NMR (CD_3OD): 2.33 (*t*, *J* = 7.0, 2 H–C(2)); 1.63 (*m*, 2 H–C(3)); 1.37 (*m*, 2 H–C(4) to 2 H–C(7)); 2.09 (*m*, 2 H–C(8), 2 H–C(17)); 5.36 (*m*, H–C(9), H–C(10), H–C(12), H–C(13), H–C(15), H–C(16)); 2.82 (*m*, 2 H–C(11), 2 H–C(14)); 0.97 (*q*, *J* = 7.5, Me). EI-MS: 295 (14, $M^{+\bullet}$), 266 (3), 252 (1), 149 (21), 109 (119), 79 (100).

Data of **9**: semisolid. $[\alpha]_D^{25} = -6.8$ (*c* = 0.2, H_2O) ([7c]: $[\alpha]_D = -7.0$ (*c* = 0.3, H_2O); [15a]: $[\alpha]_D = -8.0$ (*c* = 0.6, H_2O)). ^{1}H-NMR: data matched those in [15a]. ^{13}C-NMR (CD_3OD): 105.14 (*d*); 76.70 (*d*); 74.78 (*d*); 72.54 (*d*); 72.09 (*d*); 72.01 (*t*); 70.36 (*d*); 64.01 (*t*); 62.51 (*t*). FAB-MS (glycerol): 277 ($[M+Na]^+$).

Data of **10**: ^{1}H-NMR (CD_3OD): 5.37 (*m*, H–C(7), H–C(8), H–C(10), H–C(11), H–C(13), H–C(14)); 2.31 (*t*, 2 H–C(2)); 1.64 (*m*, 2 H–C(3)); 1.39 (*m*, 2 H–C(4), 2 H–C(5)); 2.08 (*m*, 2 H–C(6), 2 H–C(15)); 2.82 (*m*, 2 H–C(9), 2 H–C(12)); 0.97 (*q*, *J* = 7.5, 3 H–C(16)). ^{13}C-NMR (CD_3OD): 174.60 (*s*); 132.73 (*d*); 130.86 (*d*); 129.22 (*d*); 129.16 (*d*); 129.04 (*d*); 127.31 (*d*); 50.41 (*q*); 34.78 (*t*); 30.68 (*t*); 30.36 (*t*); 29.79 (*t*); 27.99 (*t*); 26.51 (*t*); 25.92 (*t*); 20.90 (*t*); 14.64 (*q*). EI-MS: 267 (14, $M^{+\bullet}$), 238 (2), 224 (2), 149 (10), 109 (12), 79 (100).

Data of **11**: ^{1}H-NMR: data in agreement with [8]. EI-MS: 264 (13, $M^{+\bullet}$), 235 (1), 233 (2), 149 (8), 121 (26), 109 (18), 107 (19), 109 (18), 79 (100).

Data of **12**: ^{1}H-NMR (CD_3OD): 2.33 (*t*, *J* = 7.0, 2 H–C(2)); 1.63 (*m*, 2 H–C(3)); 1.37 (*m*, 2 H–C(4) to 2 H–C(7)); 2.09 (*m*, 2 H–C(8), 2 H–C(17); 5.36 (*m*, H–C(9), H–C(10), H–C(12), H–C(13), H–C(15), H–C(16)); 2.82 (*m*, 2 H–C(11), 2 H–C(14)); 0.98 (*q*, *J* = 7.5, 3H–C(18)); 3.65 (*s*, MeO). EI-MS: 292 (13, $M^{+\bullet}$), 263 (3), 261 (5), 149 (15), 135 (18), 121 (26), 109 (18), 69 (20).

(Z)-2-Hydroxy-3-(4-hydroxyphenyl)prop-2-enoic Acid Methyl Ester (**13a**). ^{1}H-NMR (CD_3OD): 7.81 (*d*, *J*(2′,3′) = *J*(6′,5′) = 8.7, H–C(2′), H–C(6′)); 6.76 (*d*, *J*(3′,2′) = *J*(6′,5′) = 8.7, H–C(3′), H–C(5′)); 7.05 (*s*, H–C(3)); 3.82 (*s*, MeO). ^{13}C-NMR (CD_3OD): 137.08 (*s*, C(1′)); 134.10 (*d*, C(2′), C(6′)); 116.22 (*d*, C(3′),

C(5′)); 160.32 (*s*, C(4′)); 128.52 (*d*, C(3)); 125.36 (*s*, C(2)); 167.37 (*s*, C(1)); 52.60 (*q*, MeO). EI-MS: 194 (26, $M^{+\bullet}$), 134 (29), 107 (100). HR-EI-MS: 194.0578 ± 0.003 ($C_{10}H_{10}O_4^{+\bullet}$; calc. 194.0579).

*(*E*)-2-Hydroxy-3-(4-hydroxyphenyl)prop-2-enoic Acid Methyl Ester* (**13b**). ^{1}H-NMR (CD_3OD): 7.34 (*d*, *J*(2′,3′) = *J*(6′,5′) = 8.7, H–C(2′), H–C(6′)); 6.73 (*d*, *J*(3′,2′) = *J*(6′,5′) = 8.7, H–C(3′), H–C(5′)); 6.94 (*s*, H–C(3)); 3.72 (*s*, MeO).

*(*Z*)-3-(3,4-Dihydroxyphenyl)-2-hydroxyprop-2-enoic Acid Methyl Ester* (**14a**). UV (MeOH): 299 (8400), 219 (5200). ^{1}H-NMR (CD_3OD): 7.57 (*d*, *J*(2′,6′) = 2.0, H–C(2′)); 6.76 (*d*, *J*(5′,6′) = 8.4, H–C(5′)); 7.19 (*dd*, *J*(6′,5′) = 8.4, *J*(6′,2′) = 2.0, H–C(6′)); 7.01 (*s*, H–C(3)); 3.82 (*s*, MeO). ^{13}C-NMR (CD_3OD): 136.86 (*s*, C(1′)); 118.64 (*d*, C(2′)); 145.94 (*s*, C(3′)); 148.70 (*s*, C(4′)); 116.05 (*d*, C(5′)); 125.71 (*d*, C(6′)); 129.05 (*d*, C(3)); 125.80 (*s*, C(2)); 167.39 (*s*, C(1)); 52.35 (*q*, MeO); on selective heteronuclear decoupling of MeO (3.80 ppm), the *quint.* (*J* = 3.7) at 167.39 (C(1)) in the fully coupled ^{13}C-NMR became a *d* (*J* = 3.7). EI-MS: 210 (36, $M^{+\bullet}$), 150 (33), 123 (100). HR-EI-MS: 210.0530 ± 0.003 ($C_{10}H_{10}O_5^+$; calc. 210.0528).

*(*E*)-3-(3,4-Dihydroxyphenyl)-2-hydroxyprop-2-enoic Acid Methyl Ester* (**14b**). ^{1}H-NMR (CD_3OD): 7.34 (*d*, *J*(2′,3′) = *J*(6′,5′) = 8.7, H–C(2′), H–C(6′)); 6.73 (*d*, *J*(3′,2′) = *J*(6′,5′) = 8.7, H–C(3′), H–C(5′)); 6.94 (*s*, H–C(3)); 3.72 (*s*, MeO). ^{13}C-NMR (CD_3OD): 137.60 (*s*, C(1′)); 117.51 (*d*, C(2′)); 145.79 (*s*, C(3′)); 147.40 (*s*, C(4′)); 115.89 (*d*, C(5′)); 123.44 (*d*, C(6′)); 131.13 (*d*, C(3)); 132.35 (*s*, C(2)); 166.40 (*s*, C(1)); 52.74 (*q*, MeO); on selective heteronuclear decoupling of MeO (3.72 ppm), the *qd* (*J* = 4.2, 9.2) at 166.40 (C(1)) in the fully coupled ^{13}C-NMR became a *d* (*J* = 9.2).

Photoisomerization of 3-Aryl-2-hydroxyprop-2-enoic Acid Methyl Esters. Compound **14a** (0.30M in CD_3OD) was UV-irradiated (350 nm) in a 5-mm NMR tube for 1 h at r.t. under ^{1}H-NMR monitoring, which revealed clean equilibration with **14b** to give a mixture **14a**/**14b** 45 : 55; no by-products could be detected. The composition did not change on either subjecting the mixture to a further 1-h UV irradiation (350 nm) or allowing it to stand for 12 h at r.t. in the dark.

UV irradiation of **13b**/**14b** 1 : 2 (0.02M) under similar conditions gave **13a**/**13b** 45 : 55 and **14a**/**14b** 45 : 55. The latter mixture was separated by HPLC into the pure components.

Acetylation of **13a** *and* **14a**. Dihydroxy ester **13a** (5.0 mg, 0.025 mmol) was stirred in dry pyridine (0.6 ml) and Ac_2O (0.6 ml) at r.t. for 1 h. The mixture was then quenched with H_2O (2 ml) and extracted with AcOEt (3 × 3 ml). The org. phase was dried (Na_2SO_4) and evaporated: **15** (6.9 mg, 86%). Dihydroxy ester **14a** gave **16** under similar conditions.

Data of 2-(Acetyloxy)-3-[4-(acetyloxy)phenyl]prop-2-enoic Acid Methyl Ester (**15**): ^{1}H-NMR ($CDCl_3$): 7.59 (*d*, *J*(2′,3′) = *J*(6′,5′) = 8.7, H–C(2′), H–C(6′)); 7.12 (*d*, *J*(3′,2′) = *J*(5′,6′) = 8.7, H–C(3′), H–C(5′)); 7.27 (*s*, H–C(3)); 3.84 (*s*, MeO); 2.32, 2.30 (2*s*, AcO). ^{13}C-NMR ($CDCl_3$): 169.10 (2*s*); 162.80 (*s*); 151.51 (*s*); 136.89 (*s*); 131.28 (2*d*); 129.66 (*s*); 126.32 (*d*); 121.98 (2*d*); 52.86 (*q*); 21.16, 20.69 (2*q*). EI-MS: 278 (0.6, $M^{+\bullet}$), 247 (2), 236 (18), 194 (40), 134 (50), 43 (100).

Data of 2-(Acetyloxy)-3-[3,4-bis(acetyloxy)phenyl]prop-2-enoic Acid Methyl Ester (**16**): ^{1}H-NMR ($CDCl_3$): 7.46 (*d*, *J*(2′,6′) = 2.0, H–C(2′)); 7.20 (*d*, *J*(5′,6′) = 8.7, H–C(5′)); 7.44 (*dd*, *J*(6′,5′) = 8.7, *J*(6′,2′) = 2.0, H–C(6′)); 7.25 (*s*, H–C(3)); 3.83 (*s*, MeO); 2.31, 2.29 (1 and 2*s*, resp., AcO). ^{13}C-NMR ($CDCl_3$): 168.38 (*s*); 167.92 (2*s*); 162.71 (*s*); 142.11 (*s*); 137.57 (*s*); 130.61 (*s*); 128.28 (*d*); 125.57 (*d*); 124.80 (*d*); 123.67 (*d*); 52.72 (*q*); 20.69, 20.65, 20.59 (3*q*). EI-MS: 336 (0.5, $M^{+\bullet}$), 305 (1.3), 294 (15, $[M - CH_2{=}CO]^{+\bullet}$), 252 (44), 210 (58), 140 (58), 43 (100). HR-EI-MS: 305.0661 ± 0.003 ($C_{15}H_{13}O_7^+$ $[M - MeO]^+$; calc. 305.0661), 294.0737 ± 0.003 ($C_{14}H_{14}O_7^{+\bullet}$; calc. 294.0739).

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