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Structure elucidation and synthesis of (4*S*,5*S*,6*Z*,8*E*)-5-hydroxydeca-6,8-dien-4-olide [(*S*,*S*)-sapinofuranone B]—a novel γ-lactone metabolite of *Acremonium strictum*

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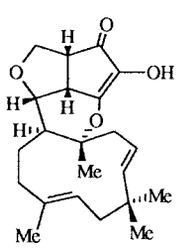
The structure of (4*S*,5*S*,6*Z*,8*E*)-5-hydroxydeca-6,8-dien-4-olide, a novel metabolite of *Acremonium strictum*, has been established by spectroscopic studies and chemical correlation with the known L-factor. The structure has been confirmed by a total synthesis in which the asymmetric centres at C-4 and C-5 were elaborated from dimethyl L-tartrate and the 6,8-diene moiety was introduced *via* Stille coupling of (*E*)-prop-1-enyltributyltin with a (*Z*)-vinylic iodide. The absolute configurations of sapinofuranones A and B, recently isolated metabolites of *Sphaeropsis sapinae*, are shown to be the corresponding (4*R*,5*S*) and (4*R*,5*R*) diastereomers of the *A. strictum* metabolite.

Xenovulene A **1** was isolated from submerged cultures of the fungus *Acremonium strictum* during a screening programme for inhibition of benzodiazepine binding to the $GABA_A$ receptor.[1] It contains an unusual polyketide-derived bicyclic cyclopentenone moiety linked *via* a furan ring to an 11-membered ring derived from the sesquiterpenoid humulene. A number of closely related metabolites have been isolated in which the cyclopentenone ring is replaced by a 6-membered phenolic ring or a 7-membered tropolone moiety. Recent biosynthetic studies[2] have shown that the cyclopentenone ring is formed by a unique ring expansion–contraction mechanism in which an intermediate methylorsellinate derivative is ring expanded to form a tropolone which is then subject to two successive contractions to form the phenol- and cyclopentenone-containing metabolites. In the course of these studies, a novel γ-lactone metabolite was isolated. We now report spectroscopic and chemical studies which allowed the assignment of the (4*S*,5*S*,6*Z*,8*E*)-5-hydroxydeca-6,8-dien-4-olide structure **2** to this metabolite.[3] The structure and stereochemistry have been confirmed by a total synthesis of **2**.

Results and discussion

In preliminary fermentation work to optimise the production of xenovulene A **1** prior to labelling studies, the growth of *A. strictum* was investigated in a fermenter instead of the normal shake flasks. No xenovulene A was produced but a novel metabolite was isolated from the fermentation extracts. This metabolite was subsequently also isolated from shake flask fermentations in which xenovulene A was present, and indeed was identified as a trace contaminant in ^{1}H NMR spectra of previously isolated samples of xenovulene A.

High resolution mass spectrometry of the new metabolite, which had an optical rotation of +19, indicated a molecular formula of $C_{10}H_{14}O_3$. The ^{1}H NMR spectrum (Table 1) showed the presence of only 13 protons, implying the presence of a free



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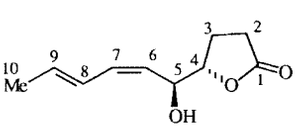

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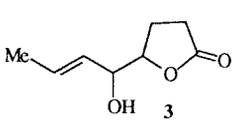

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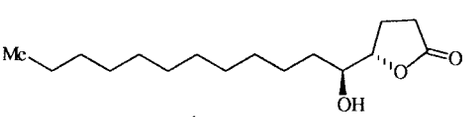

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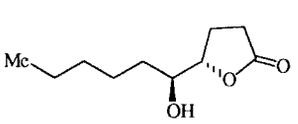

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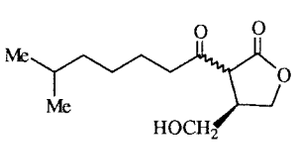

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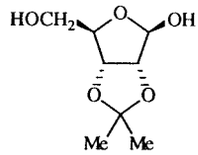
Table 1 ^{1}H and ^{13}C NMR data of **2**

Position	δ_H[a]	Multiplicity, *J*/Hz	δ_C	Multiplicity
1			180.2	s
2	2.58[b]	m	29.3	t
3	2.15	m	24.6	t
	2.35	m		
4	4.51	ddd, 7.5, 6.5, 5	85.0	d
5	4.63	ddd, 9, 5, 1.5	70.4	d
6	5.33	dddq, 11, 9, 2, 1	126.7	d
7	6.18	tm, 11	133.4	d
8	6.48	ddqd, 15, 11, 2, 1	133.6	d
9	5.86	dqt, 15, 7, 1	127.8	d
10	1.83[c]	ddd, 7, 2, 1	18.4	q

[a] ppm in $CDCl_3$. [b] 2H. [c] 3H.

hydroxy group which was confirmed by the infra-red spectrum (ν_{max} 3450 cm^{-1}). The connectivity in the ^{1}H NMR spectrum was evident from analysis of the coupling constants and was confirmed by a ^{1}H–^{1}H COSY experiment. The 10-methyl appeared as a doublet of doublets of doublets (1.83 ppm) which was clearly coupled to three of the four protons in the olefinic region. The four olefinic protons were mutually coupled, indicating a 1,3-diene system with a terminal methyl group. An (*E*,*Z*) geometry was assigned to the diene on the basis of the olefinic coupling constants of 15 and 11 Hz. The olefinic proton furthest from the methyl group was coupled to a proton at 4.63 ppm which was further coupled to one at 4.51 ppm, chemical shifts consistent with their being on oxygen-bearing carbons. The signals resulting from the remaining four protons were complex multiplets. The signal centred at 2.58 ppm integrated for two hydrogens and was coupled to that at 4.51 ppm and to the remaining two signals at 2.15 ppm and 2.35 ppm. The ^{13}C NMR spectrum (Table 1) was consistent with these observations, showing four olefinic signals, two oxygen-bearing methines, one methyl, two methylenes and a single signal in the carbonyl region at 180.2 ppm. That this was present as a γ-lactone was confirmed by the carbonyl stretch at 1772 cm^{-1} in the infra-red spectrum. These data are consistent with structure **2**.

A number of related hydroxylated γ-lactones have been reported from microbial sources. The fungal lactone **3**, which has two carbons fewer than **2**, is a metabolite of a *Nigrospora* sp.,[4] whereas muricatacin **4** from the seeds of *Annona muricata*[5] has a dodecyl side chain. L-factor **5**, which is the reduced form of **2**, was isolated from *Streptomyces griseus* and was thought to have an autoregulatory role,[6] controlling the formation of



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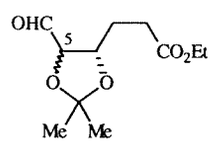
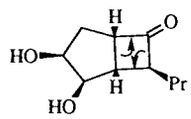


8 (5*S*)
9 (5*R*)



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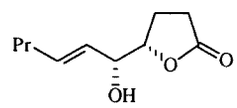

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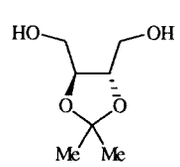

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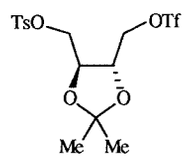

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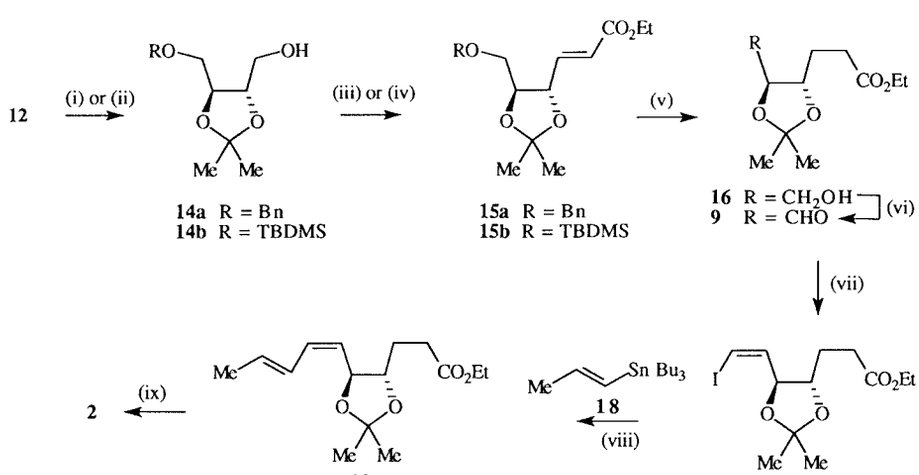
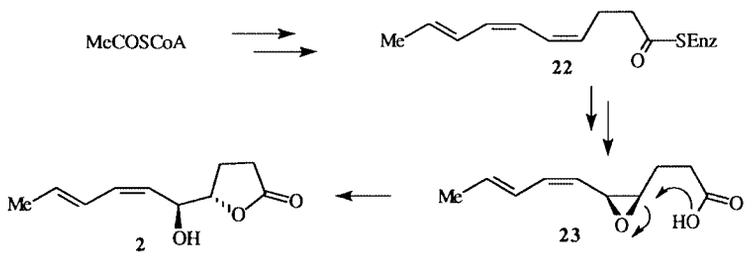
aerial mycelia and production of the anthracycline antibiotic leukaemomycin, although this activity was subsequently shown[7] to be due to contamination with trace amounts of the true regulatory molecule, A-factor **6**. The reported biological activity for L-factor **5** prompted synthetic efforts to produce all four diastereomers, and their synthesis was reported by Mori and Otsuka using Sharpless asymmetric epoxidation methdology.[8] The optical rotations of the (4*S*,5*S*) and (4*R*,5*R*) pair were +33.2 and −33.1 respectively, whereas those for the (4*S*,5*R*) and (4*R*,5*S*) diastereomers were +9.5 and −9.5 respectively.

In order to determine the absolute configuration at C-4 and C-5 in **2**, the diene was reduced by catalytic hydrogenation. The optical rotation of the saturated product was +27, which compares favourably with the value of +33.2 reported for the (4*S*,5*S*) diastereomer of L-factor **5**. Thus the (4*S*,5*S*) stereochemistry could be assigned to the new metabolite **2**, isolated from *A. strictum*.

Definitive proof for the structures and stereochemistry of **2** has been provided by a stereoselective total synthesis. It was necessary for the route chosen to introduce both the correct stereochemistry at the two asymmetric centres, and the correct geometry of the 1,3-diene in the side chain. In the earliest synthesis of L-factor,[9] the starting material was the isopropylidene-



Scheme 1 *Reagents, conditions and yields*: (i) BnCl, NaH, DMSO, 94%; (ii) TBDMSCl, NaH, DME, 86%; (iii) $Ph_3P{=}CHCO_2Et$, $C_6H_5CO_2H$, Dess–Martin periodinane, DCM, DMSO, 90%; (iv) $(COCl)_2$, DMSO, Et_3N, then $(EtO)_2POCH_2CO_2Et$, K_2CO_3, 70%; (v) H_2, 10% Pd/C, EtOH, 80%; (vi) Dess–Martin periodinane, DCM, 92%; (vii) $[Ph_3PCH_2I]^+ I^-$, NaHMDS, HMPA, 25%; (viii) $(Ph_3P)_2PdCl_2$ (10 mol%), THF, Δ_R, 57%; (ix) TsOH, MeOH, 50%.



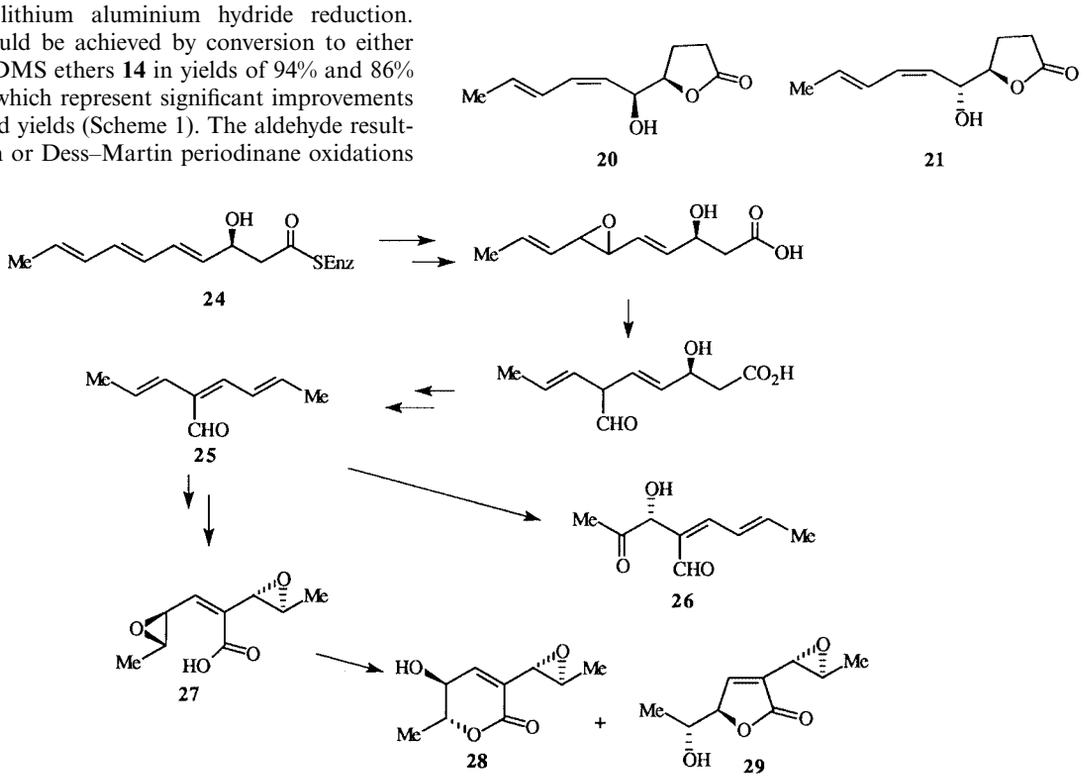
Scheme 2 Proposed biosynthesis of *A. strictum* lactone **2**.

protected ribose **7** from which both the (4*S*,5*R*) and (4*S*,5*S*) diastereomers of L-factor were elaborated *via* Wittig chain extension of the masked aldehyde, catalytic hydrogenation and periodate cleavage to give the aldehyde **8** which could be epimerised to **9**. Further Wittig chain extension, catalytic hydrogenation and deprotection gave the dihydroxy acid which spontaneously cyclises to the γ-lactone. Two more recent routes to L-factor have been reported but neither was immediately applicable to the synthesis of **2**. Roberts and co-workers[10] used an interesting photolysis of the racemic [3.2.0] bicyclic ketone **10** to give an intermediate ketene which was trapped selectively by the diol to give the racemic γ-lactone **11**. Hydrogenation of the double bond then afforded the racemic L-factor analogue. The most recent route is based on the isopropylidene-protected tartrate derivative **12**. Kotsuki *et al.*[11] converted this to the tosylate-triflate **13**. A one-pot double alkylation was used to introduce the required alkyl side chain and a propenyl group which, after ozonolysis, oxidation to the carboxylic acid and deprotection, spontaneously cyclised to give (4*S*,5*S*) L-factor.

For our synthesis, we envisaged using the aldehyde **9** for elaboration of the 1,3-diene side chain. Although this has been prepared as described above,[9] comparison of the $[\alpha]_D$ of L-factor synthesised by this route with that of the natural product indicated that the epimerisation of **8** to **9** was not completely efficient. However, Batty and Crich have described[12] the preparation of **9** in 28% overall yield from the tartrate-derived diol **12** which was used previously in the route of Kotsuki *et al.*.

Diol **12** was prepared as previously described[13] from dimethyl L-tartrate by protection of the diol as the isopropylidene acetal followed by lithium aluminium hydride reduction. Desymmetrisation could be achieved by conversion to either the benzyl[12,14] or TBDMS ethers **14** in yields of 94% and 86% respectively, both of which represent significant improvements on previously reported yields (Scheme 1). The aldehyde resulting from either Swern or Dess–Martin periodinane oxidations proved to be unstable, and so the alcohols **14** were taken through to the unsaturated esters **15** using either the one-pot procedure developed by Barrett *et al.*[15] for the *in situ* Dess–Martin oxidation of an alcohol in the presence of a stabilised phosphorus ylide (90% yield), or by immediate Wadsworth–Emmons reaction of the aldehyde formed in the Swern oxidation with triethyl phosphonoacetate (70% yield). Catalytic hydrogenation of the double bond in **15** led to concomitant removal of the protecting group, in both cases giving the desired alcohol **16** in 80% yield. Dess–Martin oxidation of the alcohol gave the aldehyde **9** in 92% yield. Based on the previously described route,[12] the above procedure has shortened the synthesis by one step and improved the overall yield of **9** from the starting diol **12** from 24 to 59%.

For elaboration of the 1,3-diene, a two-step procedure was envisaged involving the conversion of the aldehyde **9** to the (*Z*)-vinylic iodide **17** followed by Stille coupling with the (*E*)-vinylic stannane **18** which is readily prepared[16] from (*E*)-prop-1-enylbromide in 84% yield with no trace of the corresponding (*Z*)-isomer. Vinylic iodide **17** was prepared using two equivalents of the ylide generated from iodomethyltriphenylphosphonium iodide with sodium hexamethyldisilazide in the presence of HMPA.[17] After column chromatography, the (*Z*)-vinylic iodide **17** was obtained as a single geometrical isomer in a modest 25% yield. The olefinic coupling constant of 8 Hz is consistent with previously reported values for (*Z*)-vinylic iodides.[18] Stille coupling of **17** with the stannane **18** gave the diene **19** in 57% yield after chromatography. Examination of the ^{1}H NMR spectrum indicated that the 1,3-diene had the



Scheme 3 Proposed biosynthesis of aspyrone, asperlactone and avallaneol.

desired (*Z*,*E*) geometry with a coupling of 11 Hz between 6-H and 7-H, and of 14.5 Hz between 8-H and 9-H. Finally, treatment of the diene with toluene-*p*-sulfonic acid led to deprotection of the diol and spontaneous lactonisation to give **2** in 50% yield following column chromatography.

The synthetic sample had an optical rotation of +23.4 which was a little higher than the value obtained for the natural product (+19). However, the ^{1}H NMR spectrum revealed that the product was only approximately 85% pure, and was contaminated with an isomer believed to be the diene with the (*E*,*E*) geometry. Thus the product was purified by HPLC using 20% acetonitrile, 80% water and 0.01% TFA as the eluent. Analysis of the resultant material confirmed that it was pure and that it had data in good agreement with those for the natural product.

Prior to completion of this work, two closely related lactones designated sapinofuranones A and B were isolated from liquid cultures of *Sphaeropsis sapinae* and the structures determined by spectroscopic methods.[19] Sapinofuranones A and B have reported optical rotations of +65.9 and −18.9 respectively. The stereochemistries at C-5 were designated as *S* and *R* respectively by ^{1}H NMR studies of the derived Mosher's esters, but the C-4 stereochemistry was not determined. Comparison of these data with those for the *A. strictum* metabolite **2** indicated that sapinofuranone B must be the (4*R*,5*R*) enantiomer **21** and thus sapinofuranone A must be the (4*R*,5*S*) diastereomer **20**. The *A. strictum* lactone may therefore be described as (4*S*,5*S*)-(+)-sapinofuranone B.

Lactone **2** is clearly of polyketide origin and it can be proposed to be biosynthesised (Scheme 2) *via* the pentaketide-derived (*Z*,*Z*,*E*)-triene **22**. Epoxidation and opening of the epoxide **23** by the free acid formed on release from the enzyme would give the γ-lactone with inversion of stereochemistry at C-4. A number of related pentaketide metabolites are known. The pentaketide metabolites aspyrone **28** and asperlactone **29** from *Aspergillus melleus* have been shown[20] to be derived *via* a similar pentaketide-derived triene **24** which undergoes epoxidation followed by rearrangement and decarboxylation to give aldehyde **25**, which can be readily converted to avallaneol **26**, an acyclic metabolite of *Hypocrea avallaneae* (Scheme 3).[21] Aldehyde **25** can also be converted to the epoxy acid **27** which cyclises to give either the δ-lactone **28** or γ-lactone **29**.

Experimental

General methods

Nuclear magnetic resonance (NMR) spectra were recorded as solutions in deuteriochloroform unless stated otherwise, using tetramethylsilane as the internal reference on a JEOL GX270 MHz, GX400 MHz or Λ300 MHz spectrometer. The chemical shift values for all spectra are given in parts per million with coupling constants in hertz (Hz). Mass spectra (both high and low resolution) were recorded on a Fisons Autospec mass spectrometer and were obtained by electron impact ionisation (EI) or by chemical ionisation (CI) techniques. Infra-red spectra were recorded on a Perkin-Elmer 881 spectrophotometer either as liquid films on sodium chloride plates unless otherwise stated or as Nujol mulls. Optical rotations were determined as solutions, irradiating with the sodium D line ($\lambda = 589$ nm) using a Perkin-Elmer 241 MC polarimeter. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Flash column chromatography was carried out according to the procedure outlined by Still *et al.*[22] using Merck Kieselgel 60 silica gel. Routine analytical thin-layer chromatography was performed on Merck DC-Alufolien Kieselgel 60 F_{254} aluminium-backed plates. The plates were developed with the appropriate solvent system and visualised either by a Mineralight UV lamp or by dipping into a potassium permanganate solution in aqueous sodium hydroxide and heating with a hot air gun.

Culture methods

Method for the preparation of conidial suspension for storage. *Acremonium strictum* X06/15/458 was supplied by Xenova Discovery Ltd. on a slope of half-strength potato dextrose agar. A sterile aqueous solution containing 10% glycerol, 0.1% Tween 80 (10 ml) was added to a plate of well grown *A. strictum* and the surface of the plate scraped gently with a loop. After trituration with a pipette to suspend the conidia the suspension was filtered through non-absorbent cotton wool. The suspension was checked microscopically for the presence of hyphal fragments. Aliquots (1.5 ml) were placed in cryovials and frozen in liquid nitrogen.

Seed culture. Glucose (10 g l^{-1}), glycerol (15 g l^{-1}), soya peptone (Sigma; 15 g l^{-1}), NaCl (3 g l^{-1}), malt extract (Oxoid; 5 g l^{-1}), Junlon PW110 (1 g l^{-1}) and Tween 80 (1 ml l^{-1}) were dissolved in the requisite volume of deionised water. The pH was adjusted to 6.0 and the medium dispensed into flasks (100 ml per 500 ml Erlenmeyer flask) with a foil-covered cotton wool plug. Following sterilisation each flask was inoculated with conidial suspension from a cryovial (1 ml) and incubated for 3–4 days (25 °C, 240 rpm).

Isolation of (4*S*,5*S*,6*Z*,8*E*)-5-hydroxydeca-6,8-dien-4-olide 2

A seed culture of A. *strictum* was prepared according to the standard procedure and used as the inoculum (4% v/v) for standard production medium: maltose (45 g l^{-1}), yeast extract (LabM; 6.9 g l^{-1}) and Tween 80 (1 ml l^{-1}) (1.5 l), with the addition of Sigma Antifoam (1 ml l^{-1}), which had been prepared and autoclaved in a 2 l fermenter. The culture was incubated at 26 °C (500 rpm; 0.5% v/v airflow) for 8 days, then centrifuged and the liquor extracted with ethyl acetate–hexane 1 : 1 (2 × 1 l). The evaporated extract was purified by flash column chromatography (ethyl acetate–hexane 1 : 1) to afford **2** as a clear oil (23 mg); (R_f 0.20 in the same solvent system) (Found: M^+, 182.0946. $C_{10}H_{14}O_3$ requires *M*, 182.0943); $[a]_D^{20}$ +19 (*c* 0.77, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3424 (br, OH) and 1772 (CO); δ_H (300 MHz; CD_3OD) 1.83 (3H, dd, *J* 7 and 1.5, 10-H_3), 2.24 (1H, m, 3-H*H*), 2.25 (1H, m, 3-*H*H), 2.58 (2H, m, 2-H_2), 4.52 (1H, ddd, *J* 7.5, 6.5 and 5, 4-H), 4.63 (1H, ddd, *J* 9, 5 and 1, 5-H), 5.33 (1H, td, *J* 10.5 and 1, 6-H), 5.85 (1H, dq, *J* 15 and 7, 9-H), 6.18 (1H, t, *J* 10.5, 7-H) and 6.44 (1H, ddm, *J* 15, 11, 8-H); δ_C (75 MHz; CD_3OD) 18.4 (q, C-10), 24.6 (t, C-3), 29.3 (t, C-2), 70.4 (d, C-5), 85.0 (d, C-4), 126.7 (d, C-6), 127.8 (d, C-9), 133.4 (d, C-7), 133.6 (d, C-8) and 180.2 (s, C-1); *m*/*z* (EI) 183 (MH^+, 4%), 182 (M^+, 2), 164 (11), 146 (6), 118 (7) and 98 (100).

(4*S*,5*S*)-5-Hydroxydecan-4-olide 5

5% Palladium on carbon (5 mg) was added to a solution of the diene **2** (15 mg, 8×10^{-5} mol) in MeOH (5 ml). The solution was stirred rapidly under a positive pressure of H_2 until the uptake of gas had ceased. The reaction mixture was filtered through Celite and evaporated to give a clear oil, which was purified by column chromatography (ethyl acetate–light petroleum 3 : 7) to afford the lactone **5** (6 mg, 36%); $[a]_D^{17}$ +27.1 (*c* 0.55, $CHCl_3$) [lit.[8] $[a]_D^{21}$ +33 (*c* 1.11, $CHCl_3$)]; δ_H (400 MHz; $CDCl_3$) 0.89 (3H, t, *J* 7, 10-H_3), 1.25–1.45 (5H, m), 1.45–1.62 (3H, m), 2.15 (1H, ddd, *J* 12.5, 10 and 7.5, 2-H*H*), 2.25 (1H, ddd, *J* 12.5, 9.5 and 5.5, 2-*H*H), 2.45–2.75 (2H, m), 3.58 (1H, dt, *J* 8.5 and 4, 5-H) and 4.45 (1H, ddd, *J* 7.5, 7.5 and 4, 4-H); δ_C (100 MHz; $CDCl_3$) 14.0 (C-10), 22.5, 24.1, 25.1, 28.7, 31.7, 32.9, 73.7 (C-5), 82.9 (C-4) and 177.9 (C-1).

(4*S*,5*S*)-4-Benzyloxymethyl-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane 14a

The procedure of Batty and Crich was followed:[12] a suspension of sodium hydride (60% suspension in paraffin oil; 0.07 g, 1.7

mmol) in dry dimethyl sulfoxide (5 ml) was stirred under an atmosphere of nitrogen for 0.5 h. A solution of the diol **12**[13] (250 mg, 1.5 mmol) in dry dimethyl sulfoxide (1 ml) was added dropwise and the reaction mixture stirred for a further 0.5 h. Benzyl chloride (0.2 ml, 0.20 g, 1.62 mmol) was added during 15 minutes and the reaction mixture was stirred at room temperature overnight, then poured into ice–water (15 ml) and extracted with diethyl ether (3 × 20 ml). The organic layers were combined, washed with water (5 ml) and brine (5 ml), dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography (ethyl acetate–light petroleum 1:1) to afford the desired alcohol **14a** as a clear oil (0.36 g, 1.44 mmol, 94%); $[a]_D^{22}$ +7.3 (*c* 3.14, $CHCl_3$) [lit.[12] $[a]_D^{23}$ +8.3 (*c* 2.9, $CHCl_3$)]; ν_{max}(film)/cm^{-1} 3452 (br, OH), 2987, 2934, 2868, 1454, 1380 and 1371; δ_H (300 MHz; $CDCl_3$) 1.42 (3H, s, CH_3), 1.43, (3H, s, CH_3), 2.48 (1H, br s, O*H*), 3.5–3.8 (4H, m, 1-H_2 and 4-H_2), 3.95 (2H, m, 2-H and 3-H), 4.58 (2H, s, CH_2Ph) and 7.32 (5H, m, Ar-H).

(4*S*,5*S*)-4-*tert*-Butyldimethylsilyloxymethyl-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane 14b

Sodium hydride (1.13 g, 28.3 mmol, 60% dispersion in mineral oil) was added portionwise to a stirred solution of the diol **12** (4.17 g, 25.7 mmol) in 1,2-dimethoxyethane (80 ml) at 0 °C and the mixture was stirred for 5 minutes under a nitrogen atmosphere. *tert*-Butyldimethylsilyl chloride (4.28 g, 28.3 mmol) in 1,2-dimethoxyethane (80 ml) was added dropwise over 10 min and the reaction mixture was then stirred at room temperature for 5 h. The reaction mixture was then poured into water (100 ml) and the layers separated. The aqueous phase was extracted with ethyl acetate (3 × 100 ml), and the organic layers were combined, washed with water (100 ml), dried over sodium sulfate, filtered and concentrated *in vacuo* to give a yellow oil. The product was purified by flash chromatography eluting with 10% ethyl acetate in petroleum ether to give the title compound **14b** as a clear oil (6.0 g, 86%); $[a]_D$ +15.3 (*c* 7.5, $CHCl_3$) [lit.[23] $[a]_D$ +16.1 (*c* 2.4, $CHCl_3$)]; δ_H (270 MHz; $CDCl_3$) 0.01 (6H, s, $Si(CH_3)_2$), 0.90 (9H, s, $C(CH_3)_3$), 1.40 (3H, s, CH_3), 1.41 (3H, s, CH_3), 2.00 (1H, br s, OH), 3.50–3.95 (6H, m, 2 × CH_2 and 2 × CH); *m*/*z* (CI) 261 ([MH]$^+$ − CH_4, 3%), 229 (22), 219 (14), 159 (14), 131 (16), 115 (20), 107 (20) and 85 (100).

Ethyl (2*E*,4*S*,5*S*)-6-benzyloxy-4,5-isopropylidenedioxyhex-2-enoate 15a

The method of Barrett and co-workers was followed:[15] the alcohol **14a** (50 mg, 0.2 mmol), ethyl triphenylphosphoranylideneacetate (0.28 g, 0.8 mmol) and benzoic acid (98 mg, 0.8 mmol) were dissolved in dry dichloromethane (5 ml) and dry dimethyl sulfoxide (0.8 ml) at room temperature under an atmosphere of nitrogen. Dess–Martin periodinane (0.20 g, 0.48 mmol) was added in one portion and the solution immediately turned yellow. After 45 minutes stirring at room temperature the reaction was quenched by addition of saturated $NaHCO_3$ solution (10 ml), diethyl ether (10 ml) and solid $NaHCO_3$ (10 mg). After stirring for 10 minutes the mixture was filtered through Celite, the organic layer separated and the aqueous layer extracted with further diethyl ether (10 ml). The combined organic layers were dried ($MgSO_4$) and evaporated to give an orange oil, which was purified by column chromatography (light petroleum–ethyl acetate 5:1) to afford the desired alkene **15a** as a clear oil (57 mg, 0.18 mmol, 90%); ν_{max}(film)/cm^{-1} 2985, 2936, 2903, 2870, 1722 (CO), 1658, 1371 and 1302; δ_H (300 MHz; $CDCl_3$) 1.29 (3H, t, *J* 7, CH_2CH_3), 1.43 (3H, s, CH_3), 1.46 (3H, s, CH_3), 3.63 (2H, d, *J* 5, 6-H_2), 3.95 (1H, dt, *J* 8.5 and 5, 5-H), 4.23 (2H, q, *J* 7, CH_2CH$_3$), 4.44 (1H, ddd, *J* 8.5, 5.5 and 1.5, 4-H), 4.6 (2H, s, CH_2Ph), 6.1 (1H, dd, *J* 16 and 1.5, 2-H), 6.9 (1H, dd, *J* 16 and 5.5, 3-H) and 7.3 (5H, m, Ar-H).

Ethyl (2*E*,4*S*,5*S*)-6-*tert*-butyldimethylsilyloxy-4,5-isopropylidenedioxyhex-2-enoate 15b

A solution of dimethyl sulfoxide (1.09 ml, 15.4 mmol) in dichloromethane (5 ml) was added dropwise to a stirred solution of oxalyl chloride (0.66 ml, 7.6 mmol) in dichloromethane (5 ml) at −78 °C. After 15 min a solution of alcohol **14b** (1.06 g, 3.84 mmol) in dichloromethane (5 ml) was added dropwise and the mixture was stirred for 1 h at −78 °C. Triethylamine (3.20 ml, 23.0 mmol) was added dropwise and the reaction mixture was allowed to warm to room temperature. Water (20 ml) was then added and the layers were separated. The aqueous phase was extracted with dichloromethane (3 × 20 ml) and the combined organic layers were dried over sodium sulfate, filtered and concentrated *in vacuo* to give the aldehyde as a yellow oil. A mixture of the aldehyde (4.58 g, 16.7 mmol), triethyl phosphonoacetate (8.24 g, 36.8 mmol) and potassium carbonate (5.53 g, 38.0 mmol) in dichloromethane (100 ml) was stirred for 14 h at room temperature. Water (100 ml) was added and the layers separated. The aqueous phase was extracted with ethyl acetate (3 × 100 ml). The organic layers were combined, washed with water (70 ml), dried over sodium sulfate, filtered and concentrated *in vacuo* to give a pale yellow oil. Purification by flash column chromatography eluting with 10% ethyl acetate in petroleum ether gave ester **15b** (1.00 g, 70% over two steps) as a pale yellow oil; $[a]_D^{25}$ −2.2 (*c* 10.2, $CHCl_3$) [lit.[24] $[a]_D^{25}$ −2.6 (*c* 1.0, $CHCl_3$)]; δ_H (270 MHz; $CDCl_3$) 0.08 (6H, s, $Si(CH_3)_2$), 0.90 (9H, s, $C(CH_3)_3$), 1.29 (3H, t, *J* 7.0, OCH_2CH_3), 1.35 (3H, s, CH_3), 1.36 (3H, s, CH_3), 3.72 (3H, m, 5-H and 6-H_2), 4.13 (2H, q, *J* 7.0, OCH_2CH$_3$), 4.44 (1H, m, 4-H), 6.05 (1H, dd, *J* 15.5 and 1.5, 2-H), 6.87 (1H, dd, *J* 15.5 and 5.0, 3-H); *m*/*z* (EI) 329 (M$^+$ − CH_3, 14%), 299 (6), 281 (14), 241 (5), 229 (100) and 117 (83).

Ethyl (4*S*,5*S*)-6-hydroxy-4,5-isopropylidenedioxyhexanoate 16

(a). 5% Palladium on charcoal (0.5 g) was added in one portion to a solution of the unsaturated ester **15b** (1.5 g, 4.40 mmol) in ethanol (120 ml). The mixture was stirred under a hydrogen atmosphere at room temperature for 15 h. The reaction mixture was then filtered through Celite and the filtrate was concentrated *in vacuo.* The resultant yellow oil was purified by flash chromatography eluting with 40% ethyl acetate in petroleum ether to give alcohol **16** (0.80 g, 80%) as a clear oil; $[a]_D^{25}$ −19.5 (*c* 2.9, $CHCl_3$), [lit.[12] $[a]_D^{23}$ −24.1 (*c* 2.6, $CHCl_3$)]; δ_H (300 MHz; $CDCl_3$) 1.27 (3H, t, *J* 7.0, OCH_2CH_3), 1.34 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.75–2.05 (3H, m, 3-H_2 and OH), 2.46 (1H, ddd, *J* 16.5, 8.5 and 7.0, 2-*H*H), 2.52 (1H, ddd, *J* 16.5, 9.0 and 6.0, 2-H*H*), 3.64 (1H, dd, *J* 11.5 and 4.0, 6-*H*H), 3.73–3.84 (2H, m, 4-H and 6-H*H*), 3.92 (1H, td, *J* 8.0 and 4.0, 5-H), 4.13 (2H, q, *J* 7.0, OCH_2CH$_3$); *m*/*z* (EI) 217 (M$^+$ − CH_3, 78%), 201 (9), 183 (20), 171 (18), 143 (61), 131 (40), 111 (100) and 101 (63).

(b). The procedure of Batty and Crich was followed:[12] a solution of the alkene **15a** (0.50 g, 1.6 mmol) in ethanol (20 ml) was stirred vigorously with palladium on charcoal (5%, 50 mg) under an atmosphere of hydrogen for 18 h. The reaction mixture was filtered through Celite and evaporated to give a clear oil, which was purified by dry flash chromatography (gradient elution, from 100% light petroleum to 7:3 light petroleum–diethyl ether) to give the desired alcohol **16** as a clear oil (0.27 g, 1.2 mmol, 76%).

Ethyl (4*S*,5*R*)-4,5-isopropylidenedioxy-6-oxohexanoate 9

Dess–Martin periodinane (0.10 g, 0.24 mmol, 1.1 equivalents) was added to a stirred solution of the alcohol **16** (51 mg, 22 mmol) in dry dichloromethane (3 ml) under an atmosphere of nitrogen. After 2 hours' stirring at room temperature the reaction mixture was diluted with diethyl ether (10 ml) and poured into a saturated aqueous solution of $NaHCO_3$ (10 ml) contain-

ing $Na_2S_2O_3$ (2.5 g). The mixture was stirred for 5 minutes then further diethyl ether (10 ml) was added and the layers were separated. The organic layer was washed with saturated $NaHCO_3$ solution (10 ml) and water (10 ml), dried ($MgSO_4$) and evaporated to give the aldehyde **9** as a clear oil (47 mg, 0.20 mmol, 92%); ν_{max}(film)/cm^{-1} 1732 (CO) and 1718 (CO); δ_H (270 MHz; $CDCl_3$) 1.20 (3H, t, *J* 7, CH_2CH_3), 1.34 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.80 (2H, m, 3-H_2), 2.40 (2H, m, 2-H_2), 3.80 (1H, m, 4-H), 4.05 (1H, m, 5-H), 4.09 (2H, q, *J* 7, CH_2CH_3) and 9.65 (1H, d, *J* 2, CHO).

Ethyl (4*S*,5*S*,6*Z*)-4,5-isopropylidenedioxy-7-iodohept-6-enoate 17

Tetrapropylammonium perruthenate (25 mg, 5 mol%) was added in one portion to a stirred suspension of alcohol **16** (0.332 g, 1.4 mmol), *N*-methylmorpholine *N*-oxide (0.25 g, 2.1 mmol) and 5 Å molecular sieves (0.5 g) in dichloromethane (20 ml) under a nitrogen atmosphere. After 1.5 h the reaction mixture was filtered through a short pad of silica eluting with a 50% solution of ethyl acetate in petroleum ether. The filtrate was concentrated *in vacuo* at room temperature to give the aldehyde **9** (0.260 g, 77%) as a clear oil. The aldehyde was used in the next step without further purification. 2 M Sodium hexamethyldisilazide in tetrahydrofuran (2.26 ml, 4.52 mmol) was added to a suspension of the iodomethyltriphenylphosphonium iodide (2.40 g, 4.52 mmol) in dry tetrahydrofuran (30 ml). The mixture was stirred at room temperature for 15 min and the yellow suspension became a deep red solution. The reaction was cooled to −78 °C and hexamethylphosphoramide (1.5 ml) was added. The solution was stirred at −78 °C for 15 min and a solution of the aldehyde (0.26 g, 1.13 mmol) in dry tetrahydrofuran (10 ml) was added slowly dropwise. The solution was quenched with petroleum ether (5 ml) at −78 °C after 2 h and water (50 ml) was added. The mixture was extracted with ethyl acetate (3 × 50 ml), dried over sodium sulfate, filtered through a pad of silica eluting with ethyl acetate to remove remaining hexamethylphosphoramide and concentrated *in vacuo*. The crude reaction mixture was purified by dry flash column chromatography eluting with 10% ethyl acetate in petroleum ether to give the title compound **17** (0.100 g, 25%) as a pale yellow oil; $[a]_D^{25}$ +22.7 (*c* 0.9, $CHCl_3$); ν_{max}(film)/cm^{-1} 2985 (CH), 1736 (CO), 1647 (C=C); δ_H (300 MHz; $CDCl_3$) 1.26 (3H, t, *J* 7.0, OCH_2CH_3), 1.40 (3H, s, CH_3), 1.43 (3H, s, CH_3), 1.92 (1H, dtd, *J* 14.0, 8.0 and 6.5, 3-*H*H), 2.05 (1H, dddd, *J* 14.0, 9.0, 7.0 and 4.0, 3-H*H*), 2.47 (1H, ddd, *J* 16.5, 8.0 and 7.0, 2-*H*H), 2.53 (1H, ddd, *J* 16.5, 9.0 and 6.5, 2-H*H*), 3.82 (1H, td, *J* 8.0 and 4.0, 4-H), 4.14 (2H, q, *J* 7.0, OCH_2CH_3), 6.25 (1H, t, *J* 8.0, 6-H), 6.57 (1H, dd, *J* 8.0 and 1.0, 7-H); δ_C (75 MHz; $CDCl_3$) 14.3 (OCH_2CH_3), 26.9 (C-3), 27.0, 27.3 (each CH_3), 30.6 (C-2), 60.5 (OCH_2CH_3), 79.2 (C-4), 82.6 (C-5), 85.9 (C-7), 109.6 (OCO), 137.9 (C-6), 173.0 (C-1); *m*/*z* (EI) 339.0092 ($M^+ - CH_3$. $C_{11}H_{16}O_4I$ requires 339.0093, 15%), 325 (2), 297 (8), 251 (80), 183 (8), 167 (10), 149 (8), 124 (15), 115 (12) and 97 (100).

Stille coupling of vinylic iodide 17 and stannane 18

Stannane **18** (0.166 g, 0.56 mmol) in dry tetrahydrofuran (1 ml) was added to dichlorobis(triphenylphosphine)palladium(II) (0.020 g, 10 mol%) in dry tetrahydrofuran (20 ml). Iodide **17** (0.100 g, 0.28 mmol) in dry tetrahydrofuran (1 ml) was added and the reaction mixture was heated under reflux for 3 h. The mixture was cooled to room temperature and concentrated *in vacuo*. The crude mixture was purified by flash column chromatography to afford diene **19** (0.040 g, 57%) as a pale yellow oil which was judged to be approximately 85% pure by ^{1}H NMR spectroscopy; $[a]_D^{25}$ −12.5 (*c* 3.4, $CHCl_3$); ν_{max}(film)/cm^{-1} 2985 (CH), 1735 (CO), 1657 (C=C); major compound δ_H (300 MHz; $CDCl_3$) 1.25 (3H, t, *J* 7.0, OCH_2CH_3), 1.40 (6H, m, 2 × CH_3), 1.80 (3H, m, 10-H_3), 1.75–2.05 (2H, m, 3-H_2), 2.33–2.54 (2H, m, 2-H_2), 3.69 (1H, m, 4-H), 4.13 (2H, q, *J* 7.0, OCH_2CH_3), 4.51 (1H, m, 5-H), 5.23 (1H, dddq, *J* 11.0, 10.0, 2.0 and 1.0, 6-H), 5.80 (1H, dqt, *J* 14.5, 7.0 and 1.0, 9-H), 6.20 (1H, tm, *J* 11.0, 7-H), 6.35 (1H, ddqd, *J* 14.5, 11.0, 1.5 and 1.0, 8-H); major compound δ_C (75 MHz; $CDCl_3$) 14.2 (OCH_2CH_3), 18.3 (C-10), 26.8 (C-2 or C-3), 27.1, 27.2 (each CH_3), 30.6 (C-2 or C-3), 60.4 (OCH_2CH_3), 76.7, 80.0 (C-4 and C-5), 108.7 (OCO), 123.9, 126.1, 133.3, 134.3 (C-6, C-7, C-8, C-9), 173.1 (C-1); *m*/*z* (CI) 239.1286 ($[MH]^+ - C_2H_6$. $C_{13}H_{19}O_4$ requires 239.1283, 10%), 165 (80), 85 (65) and 59 (100).

Deprotection of diene 19 and lactonisation to 2

Diene **19** (35 mg, 0.13 mmol) was dissolved in methanol (5 ml) and a few crystals of toluene-*p*-sulfonic acid were added. The mixture was stirred at room temperature for 6 h. Sodium hydrogen carbonate was added, the mixture was filtered and the filtrate concentrated *in vacuo*. Purification by flash column chromatography afforded lactone **2** (12 mg, 50%) as a clear oil which was judged to be approximately 85% pure by ^{1}H NMR spectroscopy; $[a]_D^{25}$ +23.4 (*c* 0.9, $CHCl_3$); major compound δ_H (300 MHz; $CDCl_3$) 1.81 (3H, ddd, *J* 7.0, 1.5 and 1.0, 10-H_3), 2.00–2.35 (2H, m, 3-H_2), 2.45–2.70 (2H, m, 2-H_2), 4.48 (1H, ddd, *J* 7.5, 7.0 and 5.5, 4-H), 4.58 (1H, ddd, *J* 9.0, 5.5 and 1.0, 5-H), 5.32 (1H, dddq, *J* 11.0, 9.0, 1.5 and 1.0, 6-H), 5.85 (1H, dqt, *J* 15.0, 7.0 and 1.0, 9-H), 6.18 (1H, tm, *J* 11.0, 7-H), 6.35 (1H, ddqd, *J* 15.0, 11.0, 2.0 and 1.0, 8-H); major compound δ_C (75 MHz; $CDCl_3$) 18.4 (C-10), 23.7 (C-3), 28.5 (C-2), 70.1 (C-5), 82.9 (C-4), 123.9 (C-6), 126.1 (C-8), 133.9 (C-7), 134.0 (C-9), 177.1 (C-1); *m*/*z* (EI) 165.0915 ($[MH]^+ - H_2O$. $C_{10}H_{13}O_2$ requires 165.0916, 7%), 137 (8), 119 (7), 115 (30) and 85 (100).

Lactone **2** was further purified by HPLC on a Waters Nova-Pak C_{18} 8 × 100 mm radially compressed column using an isocratic elution of 20% acetonitrile, 80% water and 0.01% TFA, flow rate 2 ml min^{-1}. The UV absorbance was monitored at 210 and 254 nm using a Waters 996 PDA detector. Analysis of the product **2** by standard gradient confirmed that it was pure. Found M^+ 182.0948. $C_{10}H_{14}O_3$ requires *M* 182.0943; spectroscopic data as for the natural product.

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