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HYBRIDALACTONE, AN UNUSUAL FATTY ACID METABOLITE FROM THE RED ALGA *LAURENCIA HYBRIDA* (RHODOPHYTA, RHODOMELACEAE)

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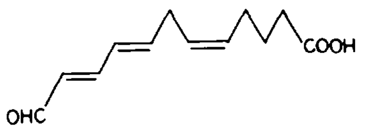
Abstract—The structure of hybridalactone (**10**) an unusual C_{20} fatty acid-derived metabolite from the red alga *Laurencia hybrida* has been assigned on the basis of spectroscopic data and results of chemical degradation experiments.

Extracts of the red alga *Laurencia hybrida* have been reported to possess antimicrobial properties[1,2] and in a preceding report the isolation and characterisation of the two active constituents 11-formyl-undeca-5(*Z*), 8(*E*), 10(*E*)-trienoic acid (**1**) and 9-hydroxy-eicosa-2(*Z*), 5(*Z*), 7(*E*), 11(*Z*), 14(*Z*), pentaenoic acid (**2**) were described.[3] During the isolation of **1** and **2** a third fatty acid-derived metabolite with unusual structural features was obtained.



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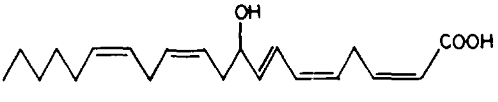

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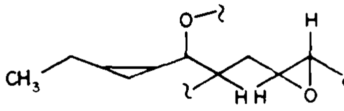
The structure of this compound has now been assigned on the basis of chemical degradation and spectroscopic data.[4]

Hybridalactone was isolated from the methanol Soxhlet extract obtained from fresh *L. hybrida*. The extract was chromatographed on florisil, the compound eluting with 7% ether in hexane. Final purification was achieved by HPLC to yield a white solid. Attempts to recrystallise the compounds have so far been unsuccessful owing to its high solubility in most organic solvents. The use of aqueous methanol also proved unsuccessful, the compound separating out as an oil.

The optically active compound $[\alpha]_D^{20} = -56°$ (MeOH, $c = 4.9$) gave IR spectral bands at 1736, 1240, 1213, 842 and 746 cm^{-1} assigned to a saturated ester and an oxygen heterocycle, while the UV spectrum showed only end absorption. The highest ion in the mass spectrum was observed at *m/e* 316 having a molecular formula analysed by high resolution mass spectrometry as $C_{20}H_{28}O_3$; from which it was concluded that the molecule contained seven degrees of unsaturation. Resonances for twenty carbon atoms were observed in the ^{13}C NMR spectrum. The lowest field signal at 173.0 ppm confirmed the presence of a saturated ester carbonyl group while the resonances at 60.82 and 58.67 ppm were assigned to the carbons of an oxirane ring. Since no absorption characteristic of an OH group was observed in the IR spectrum it was concluded that the ester was cyclic and that the C atom giving rise to a signal observed as a doublet at 78.85 ppm carried the O atom which completed the ring. The spectrum also demonstrated that the molecule possessed four olefinic carbons, though the overlap of the signals in the off-resonance spectrum did not permit assignment of their substitution pattern. Five of the seven degrees of unsaturation are accounted for by these data.

Chemical shifts and coupling constants obtained from the C_6D_6 ^1H NMR spectrum of the metabolite are summarised in Table 1, together with key decoupling data. Sequential decoupling starting with the Me triplet at 0.98 δ led to the assignment of partial structure **5**. The coupling constants J_{cis} for cyclopropanes are generally larger than J_{trans}[4] and the coupling constant J_{cf} of 8 Hz compared to 5.5 and 8 Hz between H_c and H_f and the geminal cyclopropane protons defined a *cis* ring substitution pattern. The triplet at 5.09 δ assigned to the proton H_g was coupled to the cyclopropane proton H_f and the triplet at 1.96 δ, which was in turn coupled to one of the two geminal protons at 1.53 and 2.08 δ. Irradiation of the oxirane ring proton at 3.13 δ reduced the second oxirane ring proton at 3.03 δ to a singlet, removed a 1 Hz coupling from the proton at 1.53 δ and sharpened the geminal proton 2.08 δ. The lack of further coupling into the oxirane proton at 3.03 δ prevented partial structure **5** from being extended.



5

The signals at 2.12 and 2.32 δ assigned to the methylene protons α to a CO group, formed the AB portion of an ABMX system and a second series of decouplings (summarised in Table 1) led to the assignment of substructure **6**.

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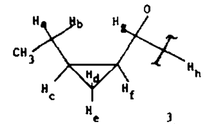
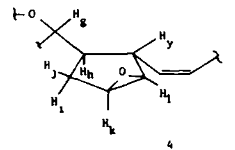
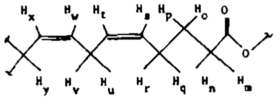
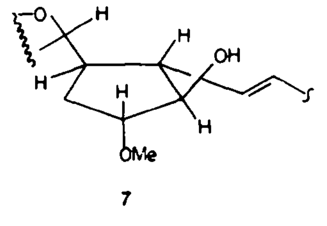
Table 1. 360 MHz ^{1}H NMR spectrum of hybridalactone (**10**) run in C_6D_6

Substructure		δ	J (Hz)	Double Resonance Experiment					
				○ Point of irradiation ← Point of effect and resulting change in multiplicity					
 3	CH_3	0.98	t J=7		○				
	H_a	1.83	qdd J=2.4,7,15	dd J=2.5,15	←	qd J=7,15	←		
	H_b	1.36	qdd J=4,7,15	dd J=4,15	←	qd J=7,15	←		
	H_c	0.68	m J=2.5,4,5.5,8,8				○		
	H_d	0.28	q J=5.5,5.5,5.5			t J=5.5,5.5	←		
	H_e	0.53	td J=5.5,8,8			dd J=5.5,8	←		
 4	H_f	0.64	tdd J=5.5,8,8,10			ddd J=5.5,8,10	←	td J=5.5,8,8	←
	H_g	5.09	t J=10,10	d J=10	←				○
	H_h	1.96	t J=10,10		○	d J=10	←	d J=10	←
	H_i	1.53	ddd J=1,10,14	dd J=1,14	←		○	dd J=10,14	←
	H_j	2.08	d J=14			s	←	sharpens	←
	H_k	3.13	dd J=1,2.5			d J=2.5	←		○
 6	H_l	3.03	d J=2.5					s	←
	H_m	2.32	ddd J=3,7.5,15	dd J=7.5,15	←	dd J=3.5,7	←		
	H_n	2.12	ddd J=3,7,15	dd J=3,15	←		○		
	H_o	1.83	part of a 2H mult.	shape changed	←	shape changed	←	shape changed	←
	H_p	1.27	m		○	7 Hz removed	←	8.5 Hz removed	←
	H_q	1.83	part of a 2H mult.	shape changed	←			shape changed	←
	H_r	2.44	m J=4.5,8.5,11,15	ddd J=4.5,11,15	←				○
	H_s	5.10	tdd J=2,4,11,11	sharpens	←	td J=4,11	←		
	H_t	5.39	tdd J=0.7,4,11,11	ddd J=0.7,4,11	←	td J=0.7,11,11	←		
	H_u	3.28	td J=11,11,14		○	t J=11,11	←	sharpens	←
	H_v	2.12	multiplet under ddd	14 Hz removed	←		○	2 Hz removed	←
	H_w	5.39	tdd J=0.7,4,11,11	ddd J=0.7,4,11	←	td J=0.7,11,11	←	ddd J=0.7,4,11	←
	H_x	4.90	td J=2,11,11	sharpens	←	t J=11,11	←		○
	H_y	3.11	d J=11					s	←

The coupling constant of 11 Hz across the double bond δ to the CO was consistent with a *cis* configuration. The stereochemistry of the second double bond could not be determined from the benzene spectrum owing to the non-first order nature of the remaining olefin signals. The NMR spectrum of the olefin region became first order on changing the solvent to chloroform and from this spectrum the second double bond was also shown to have the *cis* configuration.

Solvolysis of hybridalactone with acidic methanol at room temperature yielded a single product which, from analysis of its ^{1}H NMR spectrum, could be shown to result from methanolysis of the oxirane ring. The presence of a cyclopentane ring was confirmed as a result of irradiation experiments on **7**, which identified all of the protons in this system. Although the downfield shift of the broad double doublet at 3.70δ identified the OH as being at the homoallylic centre, the flexibility of such systems, together with the changes in magnitude of vicinal coupling arising from the orientation of the electronegative groups, prevented confirmation of the relative stereochemistry of **7**, and, therefore, of that of the epoxide in the parent compound.[5]



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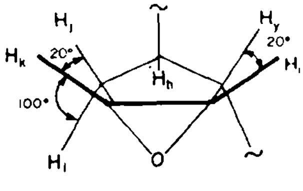
The structure assigned to hybridalactone requires coupling constants J_{hy}, J_{hj} and J_{ij} to be zero and J_{ik} and J_{ij} to be 1 Hz or smaller. Previous reports on cyclopentane oxides suggest not only that the coupling constant between the epoxide protons is smaller than usual, but also that vicinal coupling to both the cisoid and transoid protons is very small or zero.[5-7] The cyclopentene oxides suggest not only that the coupling conthe condensed oxirane ring, and of the eight conformations that arise from the four possible stereoisomers only two (**8** and **9**) are capable of producing a dihedral angle of 90° between proton H_h and the protons H_j and H_y.[8] In the case of conformer **8** the dihedral angles



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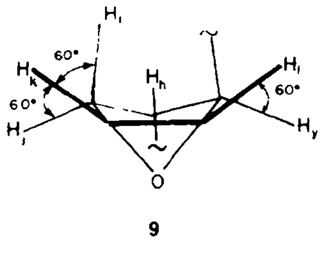

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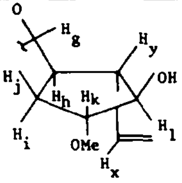
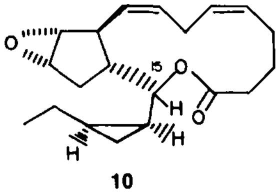
Table 2. Decoupling data of cyclopentane region of methoxy alcohol (7)

Substructure	Proton assignment δ, J (Hz)	○ Point of irradiation → Change in multiplicity		
	H_g 4.9 dd J=10,11	d J=11		
	H_h 2.18 dddd J=6,7,9,11	○		ddd J=6,9,11
	H_i 1.89 td J=7.7,15	dd J=7,15	dd J=7,15	
	H_j 1.80 ddd J=6,10,15	dd J=6,15	dd J=10,15	
	H_k 3.50 q J=7,7,7		○	
	H_l 3.70 brdd J=6,7		brd J=6	brd J=7
7	H_y 2.59 ddd J=6,7,10	dd J=7,10		○
	H_x 5.39 part of AB system			10 Hz removed

between the protons H_k, H_l and the neighbouring methylene and methine protons are ~20° and ~100° which would require J_{ki} to be small and J_{jk} and J_{ly} to be in the order of 4 Hz. On the other hand the dihedral angles made by these protons in conformer **9** are ~60° which would result in small couplings such as those observed for hybridalactone. In addition to this, conformer **8** would require proton H_j, which has zero coupling to H_h, to be a double doublet as a result of its coupling with the proton H_k whereas irradiation of proton H_k only slightly sharpens H_j.

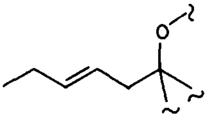
On the basis of these data it was concluded that the most probable relative stereochemistry of the substituents in the cyclopentene oxide ring in hybridalactone is all *trans* leaving only the relative stereochemistry at C_{15} unassigned. The full structure is therefore assigned as **10** or its optical antipode.



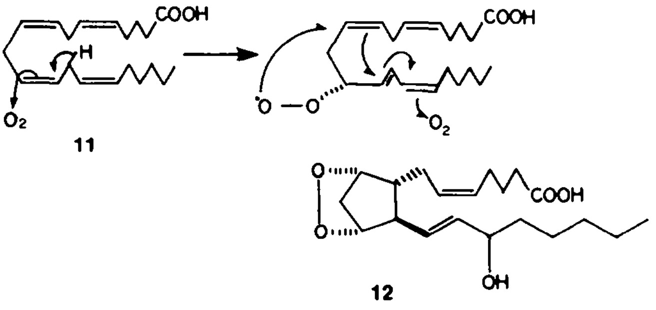
10

While the stereochemistry of the cyclopentene oxide moiety in hybridalactone is not the same as that found in the prostaglandins, e.g. PGG_2 (**12**), it may be that arachidonic acid (**11**) is a biosynthetic precursor of both structural types. A cyclisation mechanism for ring formation of hybridalactone analogous to that described for the prostaglandins[9] (Scheme 1) can be postulated though the initial oxygenation step would require removal of an allylic rather than an homoallylic proton and ring closure would be required to lead to the opposite relative side-chain stereochemistry to that found with prostaglandins (Scheme 2).

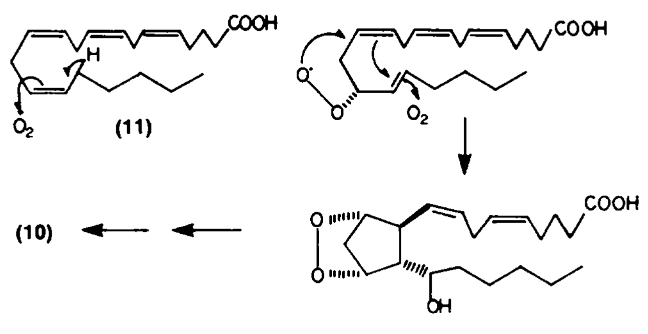
The occurrence of cyclopropane rings in fatty acid metabolites is not widespread and their formation usually involves addition of a C-1 unit *via* *S*-adenosyl methionine or ring closure *via* a cyclization mechanism. The former would require a C_{19} precursor to **11**, a fatty acid type not commonly found in algae. Further support for the C_{20} origin of hybridalactone was obtained from a second *L. hybrida* metabolite which was not fully characterised owing to the small quantity of compound isolated. The mass spectral characteristics of this compound were similar to those of **10** with a parent ion at *m/e* 316. The ^1H NMR spectrum was also smaller, although it contained no resonances assigned to cyclopropane protons. Two additional olefinic proton signals were observed and on the basis of decoupling data it was concluded that the metabolite contained partial structure **13** isomeric with the cyclopropane moiety in hybridalactone, in which the olefin has the (*E*) configuration.



(13)



Scheme 1.



Scheme 2.

EXPERIMENTAL

UV spectra were recorded on a Pye Unicam SP800 spectrophotometer and IR spectra on a Perkin–Elmer 157 spectrophotometer. Proton NMR spectra were recorded on Perkin–Elmer R12 and Brucker WH360 spectrometers using deuterobenzene and $CDCl_3$. Carbon spectra were obtained using a JEOL PFT 100 using $CDCl_3$. Low and high resolution mass measurements were obtained using a Kratos MS50 mass spectrometer.

Fresh *Laurencia hybrida* (260 g dry weight) was blended twice with MeOH to yield, on removal of the solvent, an aqueous slurry which was partitioned between $CHCl_3$ and water. The organic extract was dried over Na_2SO_4 and the solvent removed to yield a green viscous oil (1.28 g). The alga was then Soxhlet extracted with MeOH which was similarly treated to yield a dark green semi-solid extract (17.1 g).

The extract obtained from the Soxhlet (17.1 g) was fractionated on a column of silica gel (300 g, 100 × 5 cm) using a solvent gradient from 5% diethyl ether in hexane to 100% ether. Compound **10** containing only small amounts of impurity was eluted with 7% ether in hexane (80 mg) and was further purified by hplc on μ-Partisil (1 × 25 cm) eluted with 10% ether in hexane to yield a white semi-solid (74 mg). The minor component was eluted immediately after **10** as a colourless oil (2 mg) which required no further purification.

Hybridalactone **10**. Compound **10** was obtained as an optically active semi-solid, $[\alpha]_D^{20} = 56°$ (CH_3OH, $c = 0.5$); ν_{max}, (CS_2), 1736, 1213, 842, 740, 684 cm^{-1}; ^1H NMR, $CDCl_3$, δ, 0.05 (1H, q, $J = 5.5, 5.5, 5.5$ Hz), 0.57 (td. 1H, $J = 5.5, 8, 8$ Hz), 0.76 (tdd, 1H, $J = 5.5, 8, 8, 10$ Hz), 0.91 (m, 1H, $J = 2.5, 4, 5.5, 8, 8$ Hz), 1.03 (t, 3H, $J = 7$ Hz), 1.17 (ddq, 1H, $J = 2.5, 7, 13$ Hz), 1.51 (m, 1H), 1.75 (ddq, 1H, $J = 4, 7, 13$ Hz), 2.00 (m, 4H), 2.25 (d, 1H, $J = 14$ Hz), 2.27 (ddd, 1H, $J = 3, 7, 15$ Hz), 2.37 (ddd, 1H, $J = 3, 7.5, 15$ Hz), 2.38 (m, 2H), 2.97 (d, 1H, $J = 11$ Hz), 3.19 (d, 1H, $J = 2.5$ Hz), 3.38 (td, 1H, $J = 11, 11, 14$ Hz), 3.51 (dd, 1H, $J = 1, 2.5$ Hz), 4.72 (t, 1H, $J = 10$ Hz), 5.07 (td, 1H, $J = 2, 11, 11$ Hz), 5.27 (*J*dd, 1H, $J = 2, 4, 11, 11$ Hz), 5.48 (tdd, 1H, $J = 0.7, 4, 11, 11$ Hz), 5.53 (tdd, 1H, $J = 0.7, 4, 5, 11, 11$ Hz); ^1H NMR, C_6D_6, δ, 0.28 (q, 1H, $J = 5.5, 5.5, 5.5$ Hz), 0.53 (td, 1H, $J = 5.5, 8, 8$ Hz), 0.64 (tdd, 1H, $J = 5.5, 8, 8, 10$ Hz), 0.68 (m, 1H, $J = 2.5, 5.5, 8, 8$ Hz), 10.98 (t, 3H, $J = 7$ Hz), 1.27 (m, 1H, 1.36 (m, 1H, $J = 4, 7, 13$ Hz), 10.98 (t, (ddd, 1H, $J = 1, 10, 14$ Hz), 1.83 (m, 3H), 1.96 (t, 1H, $J = 10$ Hz), 2.08 (d, 1H, $J = 14$ Hz), 2.12 (dd, 1H, $J = 3, 7, 15$ Hz), 2.12 (m, 1H), 2.32 (ddd, 1H, $J = 3, 7.5, 15$ Hz), 2.44 (m, 1H, $J = 4.5, 8.5, 11, 15$ Hz), 3.03 (d, 1H, $J = 2.5$ Hz), 3.13 (dd, 1H, $J = 1, 2.5$ Hz), 3.28 (td, 1H, $J = 11, 11, 14$ Hz), 4.90 (td, 1H, $J = 2.11, 11$ Hz), 5.09 (t, 1H, $J = 10, 10$ Hz), 5.10 (tdd, 1H, $J = 2, 4, 11, 11$ Hz), 5.39 (m, 2H); ^{13}C NMR, $CDCl_3$, δ, 176.06(s), 128.88(d), 128.39(d), 128.03(d), 126.93(d), 78.79(d), 60.75(d), 58.01(d), 49.00(d), 41.56(d), 32.66(t), 27.58(t), 26.26(t), 25.72(t), 23.95(t), 23.03(t), 21.14(d), 20.35(d), 14.01(q), 8.41(t), *m/e*: 316 (0.9%, $M^{\dot{+}}$); 79 (100%), 41 (100), 91 (78), 117 (77), 67 (74), 55 (67), 105 (48), 77 (47); High resolution mass measurement: obs. 316.2038 ± 0.002; $C_{20}H_{28}O_3$ requires 316.2032.

Methanol addition product of **10**. Compound **10** (10 mg, 0.032 mmole) was added to MeOH (0.25 ml) containing sufficient diethyl ether to solubilise it. One drop of aqueous 2 M H_2SO_4 was added and the solvolysis allowed to proceed at room temp, the reaction was monitored by tlc and terminated when starting material had disappeared. The reaction was worked up by the addition of one drop of 2 M NaOH, the MeOH removed under vacuum and the products partitioned between diethyl ether and water. The organic phase was dried over $MgSO_4$ and the solvent removed to yield a yellow oil (10 mg). Purification by hplc on μ-Partisil eluted with 25% EtOAc in hexane yielded the methoxy alcohol as a colourless oil (9 mg, 82% yield). ν_{max}, (CCl_4), 3450, 1724, 1208; $[\alpha]_D^{20} = -50.1°$ ($c = 0.45$, MeOH); ^1H NMR, $CDCl_3$, δ, 0.05 (1H, q, $J = 5.5, 5.5, 5.5$ Hz), 0.59 (td, 1H, $J = 5.5, 8, 8$ Hz), 0.86 (tdd, 1H, $J = 5.5, 8, 8, 10$ Hz), 0.89 (m, 1H), 1.05 (t, 3H, $J = 7$ Hz), 1.17 (m, 1H), 1.26 (m, 1H), 1.75 (m, 1H), 1.81 (dd, 1H, $J = 7, 10$ Hz), 1.95 (m, 3H), 2.17 (tdd, 1H, $J = 5.5, 7, 10, 10$ Hz), 2.46 (dd, 1H, $J = 7, 10$ Hz), 3.15 (dt, 1H, $J = 11, 11, 14$ Hz), 3.40 (3H, s), 3.66 (AB of ABMX system, 2H), 4.76 (dd, $J = 9, 11.5$ Hz), 5.26 (brt, 1H, $J = 11, 11$ Hz), 5.29 (td, 1H, $J = 2.5, 10, 10$ Hz), 5.40 (td, 1H, $J = 2.5, 10, 10$ Hz), 5.59 (td, 1H, $J = 4, 11, 11$ Hz); *m/e* 348 (0.5%, $M^{\dot{+}}$), 41 (100%), 45 (96), 79 (81), 67 (73), 55 (64), 39 (52), 91 (50), 43 (48); High resolution mass measurement: obs. 348.2279 $C_{21}H_{32}O_4$ requires 348.2260.

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