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PAPER

New apocarotenoids and β-carotene cleavage in *Blakeslea trispora*†

Alejandro F. Barrero,*[a] M. Mar Herrador,[a] Pilar Arteaga,[a] Jesús Gil,[a] Jose-Antonio González,[a] Eugenio Alcalde[b] and Enrique Cerdá-Olmedo*[b]

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Mixed cultures of strains of opposite sex ("mated" cultures) of *Blakeslea trispora* contain trisporic acids and other apocarotenoids, some of which mediate the sexual responses of this fungus and other Mucorales. In mated cultures of the wild-type strains F986 and F921 we identified eleven apocarotenoids: two C_{18} trisporoids, three C_{15} compounds with a monocyclofarnesane skeleton, a C_{13} compound, and five C_7 compounds with a 2-methylhexane skeleton. Six of them are new natural products and two others are new for *Blakeslea*. Their structures were established by NMR and mass spectra and those of the C_7 and C_{13} compounds were confirmed by chemical synthesis. The finding of these compounds and the presence of approximately equimolecular amounts of the C_{18}, C_{15}, and C_7 families led to the conclusion that β-carotene is initially split in three fragments by cleavage of its 13,14 and 11′,12′ double bonds.

Introduction

β-Carotene (**1**, Fig. 1) is a natural pigment, antioxidant, and pro-vitamin A with many applications in the alimentary, pharmaceutical, and cosmetic industries.[1] It is obtained commercially by either chemical synthesis or biotechnology, particularly from the fungus *Blakeslea trispora* (syn. *Choanephora trispora*, Mucoromycotina, Mucorales, Choanephoraceae). The wild-type strains of this fungus belong to either the (+) or the (–) sex, and many pairs of strains of opposite sex, cultured together ("mated" cultures"), increase their β-carotene content and start the morphological program of the sexual cycle. These physiological effects were attributed to apocarotenoids such as trisporic acid C (**2**) and similar compounds present in mated cultures of *Blakeslea*.[2] The culture media of *Blakeslea* contain apocarotenoids with 18 carbons (**2–14**),[2a,3] called trisporoids, or with 15 carbons (**15–17**),[4] often called apotrisporoids because they were presumed to derive from the former.[5] On the other hand two apocarotenoids with 7 carbons (**23**, **24**) have been found recently in cultures from another Mucoral, *Phycomyces blakesleeanus*.[6a]

We have studied the apocarotenoids in cultures of our standard wild types of *B. trispora*. The structure of the identified products and their relative amounts indicate that the apocarotenoids of *B. trispora* derive from an asymmetrical double cleavage of β-carotene.

[a]*Department of Organic Chemistry, Institute of Biotechnology, University of Granada, Avda. Fuente Nueva, s/n, 18071, Granada, Spain. E-mail: afbarre@ugr.es; Fax: +34 958243318; Tel: +34 958243318*
[b]*Department of Genetic, Faculty of Biology, University of Sevilla, Reina Mercedes, s/n, 41071, Sevilla, Spain. E-mail: eco@us.es; Tel: +34 954624107*
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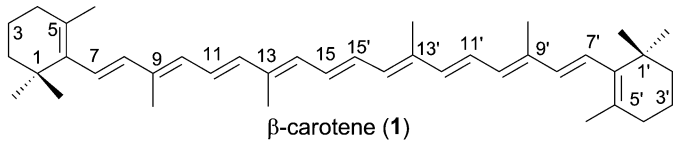
Results and discussion

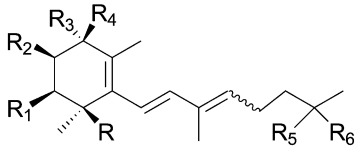
Results

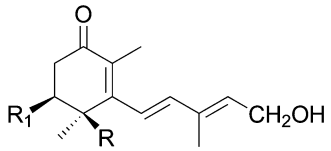
We analyzed the agar medium where the strains F986, sexually (+), and F921, sexually (–) of *B. trispora* had been cultured together for three days. A clean medium, obtained by freezing and squeezing the agar and centrifuging the resulting liquid, was brought to pH 8 with NaOH and extracted with ethyl acetate. This "neutral extract" was fractionated by semi-preparative normal-phase HPLC. The remaining water solution was brought to pH 2 with HCl and extracted with ethyl acetate. This "acid extract" was fractionated in the same way after methylation with (trimethylsilyl)diazomethane ($TMSCHN_2$).

We isolated eleven apocarotenoids: three C_{15} (**18** and **19**, as methyl esters, and **17**), two C_{18} (the 9*E* and 9*Z* isomers of **2**, both as methyl esters), five C_7 (**20–24**), and one C_{13} apocarotenoid (**25**, as methyl ester) (Fig. 2). The structures of five of them (**17**, **23**, **24**, and the 9*E* and 9*Z* isomers of **2**) were determined by comparing their spectroscopic data with those reported in the literature.[4c,6] Apocarotenoid **25** is a new natural product, but its methyl ester (**25a**) has been described as an intermediate in the synthesis of trisporic acid B.[7] The five remaining apocarotenoids (**18–22**) are new to science.

Apocarotenoid **22** is the most abundant C_7 apocarotenoid in our cultures. Its molecular formula $C_7H_{12}O_2$ was deduced from HRFABMS. Its IR spectrum showed the absorption bands of a hydroxyl group at 3447 cm^{-1} and of a conjugated diene at 1600 and 1650 cm^{-1}. The ^{13}C NMR spectrum showed seven signals: two primary alcohol signals at δ 63.6 and 68.3, a methyl signal at δ 14.1, and four signals for disubstituted and trisubstituted double bonds at δ 137.9 (C), 131.8 (CH), 127.1 (CH) and 123.8 (CH). The 2-methyl-2,4-hexadiene skeleton was established by the direct



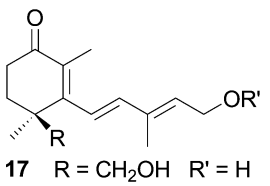




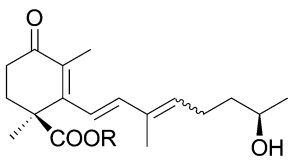
Trisporic acid C (**2**) R = COOH $R_1 = R_2 = H$ $R_3,R_4 = O$ $R_5 = H$ $R_6 = OH$
Trisporic acid A (**3**) R = COOH $R_1 = R_2 = R_5 = R_6 = H$ $R_3,R_4 = O$
Trisporic acid B (**4**) R = COOH $R_1 = R_2 = H$ $R_3,R_4 = R_5,R_6 = O$
Trisporic acid D (**5**) R = COOH $R_1 = OH$ $R_2 = H$ $R_3,R_4 = R_5,R_6 = O$
Trisporic acid E (**6**) R = COOH $R_1 = H$ $R_2 = OH$ $R_3,R_4 = O$ $R_5 = H$ $R_6 = OH$
Trisporin B (**7**) R = CH_3 $R_1 = R_2 = H$ $R_3,R_4 = R_5,R_6 = O$
Trisporin C (**8**) R = CH_3 $R_1 = R_2 = H$ $R_3,R_4 = O$ $R_5 = H$ $R_6 = OH$
Trisporol B (**9**) R = CH_2OH $R_1 = R_2 = H$ $R_3,R_4 = R_5,R_6 = O$
Trisporol C (**10**) R = CH_2OH $R_1 = R_2 = H$ $R_3,R_4 = O$ $R_5 = H$ $R_6 = OH$
Methyl 4-dihydrotrisporate B (**11**) R = COOMe $R_1 = R_2 = H$ $R_3 = H$ $R_4 = OH$ $R_5,R_6 = O$
Methyl 4-dihydrotrisporate C (**12**) R = COOMe $R_1 = R_2 = H$ $R_3 = H$ $R_4 = OH$ $R_5 = H$ $R_6 = OH$
Methyl trisporate B (**13**) R = COOMe $R_1 = R_2 = H$ $R_3,R_4 = R_5,R_6 = O$
Methyl trisporate C (**14**) R = COOMe $R_1 = R_2 = H$ $R_3,R_4 = O$ $R_5 = H$ $R_6 = OH$

Apotrisporin (**15**) R = CH_3 $R_1 = H$
Apotrisporin E (**16**) R = CH_3 $R_1 = OH$
Apotrisporol (**17**) R = CH_2OH $R_1 = H$

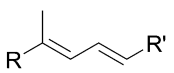
Fig. 1 Apocarotenoids isolated from cultures of different strains of *Blakeslea trispora*.



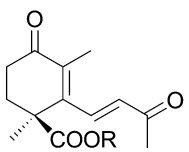
17 R = CH_2OH R' = H
18 R = COOH R' = H
18a R = COOMe R' = H
19 R = COOH R' = Ac
19a R = COOMe R' = Ac



2 R = H, 9*E*
2 R = H, 9*Z*
2a R = Me, 9*E*
2a R = Me, 9*Z*



20 R = CH_2OH R' = CHO
21 R = CHO R' = CH_2OH
22 R = R' = CH_2OH
23 R = CH_2OH R' = COOH
23a R = CH_2OH R' = COOMe
24 R = COOH R' = CH_2OH
24a R = COOMe R' = CH_2OH



25 R = H
25a R = Me

Fig. 2 Apocarotenoids isolated from mated cultures of the *B. trispora* wild-type strains F986 and F921.

coupling of the three olefinic protons in the ^{1}H NMR spectrum at δ 6.43 (dd, J_1 = 11.0 Hz, J_2 = 15.1 Hz), 6.02 (d, J = 11.0 Hz), and 5.78 (dt, J_1 = 5.5 Hz, J_2 = 15.1 Hz). The *E* stereochemistry of the disubstituted double bond was deduced from its coupling constant (15.1 Hz) and that of the trisubstituted one, from the chemical shifts at C-1 (δ 68.3) and C-7 (δ 14.1) in the ^{13}C NMR spectrum.[8] We conclude that compound **22** is (2*E*,4*E*)-2-methyl-2,4-hexadiene-1,6-diol.

Compound **20** and **21** shared most of their ^{1}H NMR signals with compound **22** (Table 1). The ^{1}H NMR spectrum of compound **20** lacked one of the CH_2OH signals (δ 4.15, d, J = 5.5 Hz) of compound **22**, but showed a CHO signal (δ 9.62, d, J = 8.0 Hz). The ^{1}H NMR spectrum of compound **21** lacked the other CH_2OH signal (δ 4.02, s) of compound **22**, but showed a CHO signal (δ 9.48, s).

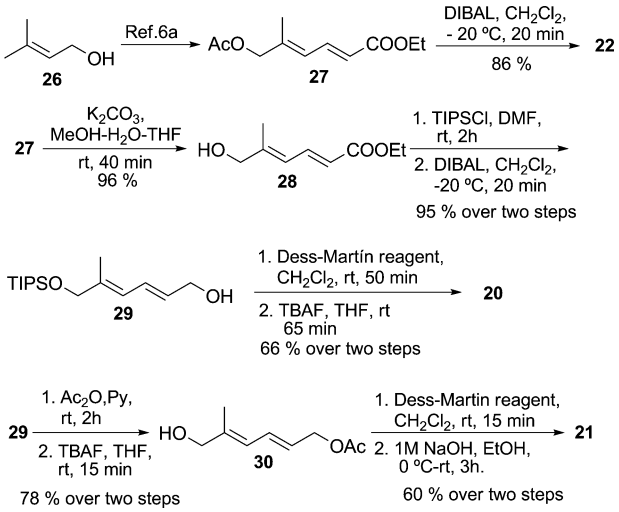
Table 1 ^{1}H and ^{13}C NMR data of **20–22**[a]

	20		**21**	**22**	
C	δ_H	δ_C	δ_H	δ_H	δ_C
1	9.62 d (8.0)	194.1	9.48 s	4.02 s	68.3
2	6.09 dd (8.0; 15.1)	121.7	—	—	137.9
3	7.65 dd (11.6; 15.1)	148.7	7.03 d (11.2)	6.02 d (11.0)	123.8
4	6.48 d (11.6)	131.7	6.85 dd (11.2, 15.1)	6.43 dd (11.0; 15.1)	131.8
5	—	152.6	6.44 dt (4.6; 15.1)	5.78 dt (5.5; 15.1)	127.1
6	4.18 br s	67.0	4.31 d (4.6)	4.15 d (5.5)	63.6
7	1.92 s	14.6	1.82	1.73	14.1

[a] δ in ppm. J in Hz in parentheses.

The proposed structures for **20–22** were confirmed by chemical synthesis (Scheme 1).

An efficient synthesis of acetoxyester **27** from the commercial prenol **26** (3-methylbut-2-en-1-ol) has been reported.[6a] Reduction of **27** with excess diisobutylaluminium hydride (DIBAL) produced **22** with a yield of 86%, whereas the chemoselective saponification of the acetoxyl group with K_2CO_3 produced the hydroxyester **28** with a yield of 96%. Protection of **28** with triisopropylsilyl chloride (TIPSCl) and reduction with DIBAL produced to **29** with a yield of 95%. Oxidation of **29** with the Dess–Martin reagent and removal of the silyl protection with tetrabutylammonium fluoride (TBAF) produced **20** with a yield of 66%. Acetylation of **29** with Ac_2O/pyridine and removal of the silyl protection with TBAF produced **30** with a yield of 78%. Oxidation of **30** with the Dess–Martin reagent and saponification with 1 M NaOH produced **21** with a yield of 60%. The spectroscopic data of the synthetic products coincided with those of the natural products.



Scheme 1 Synthesis of the apocarotenoids **20**–**22**.

Table 2 ^{1}H and ^{13}C NMR data of **17**, **18a**, **19a**[a]

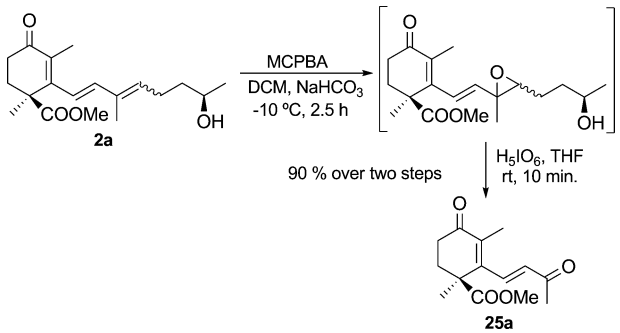
	17		**18a**		**19a**	
C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	—	41.2	—	46.9	—	47.0
2a	1.69–1.73 m	33.9	1.92–1.97 m	34.5	1.91–1.96 m	34.5
2b	2.23–2.29 m	—	2.37–2.43 m	—	2.36–2.41 m	—
3a	2.53–2.60 m	31.8	2.50–2.54 m	33.5	2.45–2.53 m	33.6
3b	2.53–2.60 m	—	2.50–2.54 m	—	2.45–2.53 m	—
4	—	199.0	—	197.4	—	197.8
5	—	135.5	—	132.5	—	133.0
6	—	157.4	—	152.3	—	152.2
7	6.22 d (16.3)	124.4	6.34 s	124.6	6.30 d (16.5)	125.2
8	6.26 d (16.3)	140.3	6.34 s	139.8	6.35 d (16.5)	139.1
9	—	135.5	—	135.9	—	137.9
10	5.76 t (6.8)	132.8	5.77 dd (1.1; 6.6)	134.1	5.67 br t (6.9)	128.7
11	4.34 d (6.8)	59.4	4.33 d (6.6)	59.6	4.72 d (6.9)	61.2
12	1.86	13.8	1.83 s	12.5	1.85 s	12.6
13a	3.44 d (11.0)	69.5	—	176.3	—	176.3
13b	3.73 d (11.0)	—	—	—	—	—
14	1.14	21.7	1.54 s	23.0	1.49 s	23.1
15	1.87	12.5	1.94 s	12.3	1.92 s	12.5
OH	1.53 br s	—	—	—	—	—
OMe	—	—	3.69 s	52.5	3.67 s	52.6
Ac	—	—	—	—	—	170.9
Ac	—	—	—	—	2.06 s	21.0

[a] δ in ppm. J in Hz in parentheses.

The molecular formula of methyl ester of apocarotenoid **18** (**18a**) was $C_{16}H_{22}O_4$ according to its HRFABMS. Its structure was established from its NMR data (Table 2), which indicated a COOMe group attached to C1, instead of the CH_2OH group of the well known apotrisporol (**17**).

The spectroscopic data of methyl ester of **19** (**19a**) led us to the conclusion that **19a** was the acetyl derivative of **18a** (Table 2).

Compound **25** is the first C_{13} apocarotenoid isolated in the Mucorales. It is found in very small amounts in the cultures and could have been formed from a C_{15} or C_{18} apocarotenoid by spontaneous oxidative breakage or a retroaldol reaction. It was identified by comparison of spectral data of its methyl ester **25a** with those published for a synthetic compound.[7] The structure was confirmed by semisynthesis (Scheme 2).



Scheme 2 Synthesis of apocarotenoid **25a**.

The reaction of the methyl ester of the trisporic acid C (**2a**) with *m*-chloroperoxybenzoic acid (MCPBA) produced the regioselective epoxidation of the 9,10 double bond. Treatment of the crude product with periodic acid produced **25a** with an overall yield of 90%. Its spectroscopic data coincided with those of the methyl ester of the natural product.

A semiquantitative analysis based on the ^{1}H NMR signals from spectra of the "neutral extract" and "acid extract" indicated that the three groups of apocarotenoids (trisporoids with 18 carbons, cyclofarnesoids with 15 carbons, and methylhexadienes with 7 carbons) were found in approximately equimolar amounts. For this summation the small amount of the only C_{13} apocarotenoid can be disregarded.

Small amounts of compounds **23** and **24** and of three other compounds were detected by their UV absorption in the chromatograms of the wild type F921, but not in those of strain SB64, a mutant derived from it and completely devoid of β-carotene. This confirms that **23** and **24** are apocarotenoids, as shown already for *Phycomyces*.[6a]

Discussion

There are four reasons to think that the compounds described here derive from β-carotene: the structural similarity between them and three segments of the β-carotene molecule (Fig. 3); the approximate equimolar amounts of trisporoids, cyclofarnesoids, and methylhexadienes; the early label experiments by Austin *et al.*,[2b] and the comparisons[6a] of wild-type strains with mutants devoid of β-carotene.

These arguments indicate that the three families of apocarotenoids of *Blakeslea* result from the double oxidative cleavage of β-carotene at its 13,14 and 11′,12′ double bonds. This mechanism was already shown for *Phycomyces*[6a] and is likely to be common to all Mucorales.

Our results with *Blakeslea* and the previous ones with *Phycomyces* argue strongly against the hypothesis[2b] that trisporic acids derive from β-carotene *via* retinal. This hypothesis was already suspicious because of the failure of the efforts to identify retinal in *Phycomyces* cultures. Our results also reject the alternative hypothesis that β-carotene is cut twice at its 13,14 and 13′,14′ double bonds to produce the first trisporoid and that C_{15} apocarotenoids result from the secondary loss of three carbon atoms of the side chain.

The two C_7 compounds with an aldehyde group and a hydroxy group **20** and **21** were the least abundant and probably represent metabolic intermediates. The likely biosynthetic pathway would

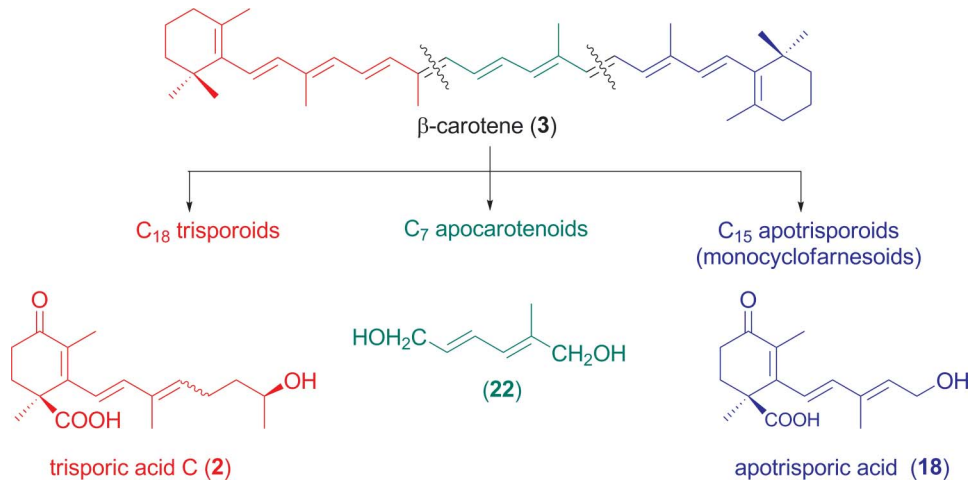


Fig. 3 The three families of apocarotenoids in *Blakeslea trispora*. According to our results, β-carotene, **1**, must be split at the points indicated by the wavy lines to produce compounds with 18 carbons (left), 7 carbons (center), and 15 carbons (right). The example given for each group is one of the compounds reported here.

start with the reduction of one end of the initial dialdehyde, not detected. *Phycomyces* oxidizes the other end to a carboxylic group, while *Blakeslea* has the additional capability to form a diol.

The metabolism of the apocarotenoids is not well fixed or conserved in the Mucorales, not even in strains assigned to the same species. Thus, we have not found fourteen apocarotenoids previously identified in other strains of *B. trispora*, but have added eight new ones to the list.

Conclusions

The Mucorales produce a large diversity of apocarotenoids, depending not only on the taxonomic species, but on the particular strains. Of the eleven apocarotenoids found in mated cultures of *B. trispora* strains F986 and F921, six are new natural products and two are new for *Blakeslea*, while fourteen other apocarotenoids reported from other strains of *Blakeslea* have not been found now. The eleven apocarotenoids include two C_{18} compounds, three C_{15} compounds, five C_7 compounds, and a small amount of one C_{13} compound. The initial step in the biosynthesis of the apocarotenoids is a double cleavage of β-carotene at its 13,14 and 11′,12′ double bonds, which gives rise to the heads of the C_{18}, C_{15}, and C_7 families of apocarotenoids. This mechanism, already seen in *Phycomyces*, is likely to be general to the Mucorales.

Experimental

General details

For general details see Polaino *et al.*[6a]

Strains and culture conditions

Strains F986 and F921 are wild-type (+) and (−) strains of *Blakeslea* (*Choanephora*) *trispora*, respectively, and were obtained from VKM (All-Russian Collection of Microorganisms, Moscow, Russia). Plates containing 25 ml minimal agar medium[9] were inoculated with 5×10^3 spores of each sex and incubated in the dark at 30 °C for three days.

Extraction and fractionation of apocarotenoids

For the extraction and fractionation of apocarotenoids see Polaino *et al.*[6a]

An initial extract of 500 mL (from 1 L of medium of mated cultures F921 × F986) yielded neutral and acid dry extracts of 114 and 246 mg, respectively.

The neutral extract (70 mg) was fractionated by semi-preparative HPLC. The fraction (10.2 < RT < 15.4 min, 5 mg) contained **20**. The fraction (15.4 < RT < 17.8 min, 20 mg) contained a 2 : 1 : 12 mixture of **20**:**21**:**22**. The fraction (17.8 < RT < 21.5 min, 5.2 mg) contained a mixture 3 : 1 mixture of **22**:**17**. The fraction (21.5 < RT < 25.4 min, 11 mg) contained **17**.

The acid fraction was methylated with $TMSCHN_2$ and then it was also fractionated by semi-preparative HPLC.

(2*E*,4*E*)-6-Hydroxy-5-methyl-2,4-hexadienal (20). Yellow syrup. ^{1}H NMR (500 MHz, $CO(CD_3)_2$): see Table 1; ^{13}C NMR (125 MHz, $CO(CD_3)_2$): see Table 1; *m*/*z* (HRMS(FAB)) 149.0580 (M + Na. $C_7H_{10}O_2Na$ requires 149.0579).

(2*E*,4*E*)-6-Hydroxy-2-methyl-2,4-hexadienal (21). Yellow syrup. ^{1}H NMR (500 MHz, $CO(CD_3)_2$): see Table 1; *m*/*z* (HRMS(FAB)) 149.0580 (M + Na. $C_7H_{10}O_2Na$ requires 149.0579).

(2*E*,4*E*)-2-Methyl-2,4-hexadiene-1,6-diol (22). Colourless syrup. ^{1}H NMR (500 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1; *m*/*z* (HRMS(FAB)) 151.0734 (M + Na. $C_7H_{12}O_2Na$ requires 151.0735).

Apotrisporol (17). Colourless syrup. It has been identified by spectroscopic data (see Table 2). These data were consistent with those previously reported.[4c]

Methylation of the acid fraction

$TMSCHN_2$ 2 M in Et_2O (232 μL) was added under stirring to a solution of the acid fraction (70 mg) in C_6H_6:MeOH (4 : 1 v/v) (2.2 mL) at 0 °C. The solution was left for 5 min at room temperature and the solvent was evaporated at low pressure to obtain a methyl

ester mixture (80 mg) that was fractionated by semi-preparative HPLC. The fraction (10.5 < RT < 13.1 min, 3.4 mg) contained **19a**. The fraction (13.4 < RT < 15.9 min, 9 mg) contained a 2 : 1 mixture of **23a** and **24a**. The fraction (16.1 < RT < 16.5 min, 9.5 mg) contained **2a**(9*E*). The fraction (16.5 < RT < 17.0 min, 3.9 mg) contained a 2 : 2 : 1 mixture of **2a**(9*E*), **2a**(9*Z*) and **25a**. The fraction (17.0 < RT < 18.4 min, 11.9 mg) contained a 5 : 1 mixture of **2a**(9*Z*) and **25a**. The fraction (18.4 < RT < 20.7 min, 10 mg) contained **2a**(9*Z*). The fraction (20.7 < RT < 23.1 min, 4 mg) contained a 2 : 1 mixture of **2a**(9*Z*) and **18a**. The fraction (23.1 < RT < 25.6 min, 6 mg) contained **18a**.

(2*E*,4*E*)-6-hydroxy-5-methylhexa-2,4-dienoic acid (23, as methyl ester) and (2*E*,4*E*)-6-hydroxy-2-methylhexa-2,4-dienoic acid (24, as methyl ester). Their spectroscopic data coincided with those previously reported.[6a]

Apotrisporic acid (18, as methyl ester). Colourless syrup. ^{1}H NMR (500 MHz, $CDCl_3$): see Table 2; ^{13}C NMR (125 MHz, $CDCl_3$): see Table 2; *m*/*z* (HRMS(FAB)) 301.1414 (M + Na. $C_{16}H_{22}O_4Na$ requires 301.1416).

Apotrisporic acid 11-acetate (19, as methyl ester). Colourless syrup. ^{1}H NMR (500 MHz, $CDCl_3$): see Table 2; ^{13}C NMR (125 MHz, $CDCl_3$): see Table 2; *m*/*z* (HRMS(FAB)) 343.1518 (M + Na. $C_{18}H_{24}O_5Na$ requires 343.1521).

(7*E*, 9*E*)-Trisporic acid C (2(9*E*)), as methyl ester). Colourless syrup. Its spectroscopic data coincided with those previously reported.[6c]

(7*E*,9*Z*)-Trisporic acid C (2(9*Z*)), as methyl ester). Colourless syrup. Its spectroscopic data coincided with those previously reported.[6b,c]

Methyl (*E*)-1,3-dimethyl-4-oxo-2-(3-oxo-1-butenyl)-2-cyclohexene-1-carboxylate (25a). Colourless syrup. Its spectroscopic data coincided with those previously reported.[7]

Preparation of 25a

m-CPBA (64 mg, 2.27 mmol) in DCM (0.8 mL) was added dropwise to a mixture of **7** (50 mg, 0.16 mmol) in dichloromethane (DCM) (2.6 mL) and 0.5 M $NaHCO_3$ (0.5 mL) under stirring and argon at 0 °C. After 2.5 h, the mixture was diluted with *t*-butyl methyl ether and washed with 0.5 M $NaHCO_3$, a saturated solution of sodium thiosulfate and brine, successively. The organic phase was dried over anhydrous Na_2SO_4 and filtered. The residue obtained (50 mg) after removing the solvent under low pressure was dissolved in THF (2.6 mL) and H_5IO_6 (35.7 mg, 0.16 mmol) was added at room temperature. After 10 min, water (10 mL) was added to the mixture and tetrahydrofuran (THF) was removed under low pressure. The aqueous phase was extracted with *t*-butyl methyl ether and the organic phase was dried over anhydrous Na_2SO_4 and filtered. The residue obtained after removing the solvent under low pressure was chromatographed in a silica gel column (hexane : *t*-butyl methyl ether, 1/1, v/v) to obtain **25** (36 mg, 90%).

Preparation of (2*E*,4*E*)-6-hydroxy-5-methyl-2,4-hexadienal (20)

Saponification of 27. Preparation of ethyl (2*E*,4*E*)-6-hydroxy-5-methyl-2,4-hexadienoate (28). K_2CO_3 (20 g) and K_2CO_3 2 M (43 mL) were added to a solution of **27** (1.4 g, 6.65 mmol) in THF (84 mL) and MeOH (25 mL). The mixture was stirred for 40 min at room temperature. Then the solvent was evaporated under low pressure to obtain a residue that was extracted with Et_2O. The organic phase was washed with brine, dried over anhydrous Na_2SO_4 and filtered. The residue obtained after removing the solvent under low pressure was chromatographed in a silica gel column (petroleum ether (bp 30–40 °C)/Et_2O, 50/50, v/v) to obtain **28** (1.08 g, 96%).

Ethyl (2*E*,4*E*)-6-hydroxy-5-methyl-2,4-hexadienoate (28). Yellow oil. IR ν_{max}(film)/cm^{-1} 3435, 1641, 1308, 1276, 1160, 1035 and 983; δ_H(300 MHz; $CDCl_3$; Me_4Si) 7.62 (1H, dd, *J* 11.7 and 15.2, H-3), 6.29 (1H, d, *J* 11.7, H-4), 5.91 (1H, d, *J* 15.2, H-2), 4.24 (2H, q, *J* 7.1, OCH_2CH_3), 4.19 (2H, s, H-6), 1.92 (3H, s, H-7), 1.72 (1H, br s, OH) and 1.33 (3H, t, *J* 7.1, OCH_2CH_3); δ_C(75 MHz; $CDCl_3$; Me_4Si) 167.5 (C, C-1), 147.2 (C, C-5), 140.0 (CH, C-3), 121.8 (CH, C-4), 121.0 (CH, C-2), 67.6 (CH_2, C-6), 60.4 (CH_2, OCH_2CH_3), 14.7 (CH_3, OCH_2CH_3)[a] and 14.4 (CH_3, C-7)[a] ([a]Signals with the same letter are exchangeable); *m*/*z* (HRMS(FAB)) 193.0844 (M + Na. $C_9H_{14}O_3Na$ requires 193.0841).

Preparation of hydroxy-ether 29. Imidazole (1.07 g, 15.73 mmol) and TIPSCl (1.45 g, 7.42 mmol) were added to a solution of **28** (1.07 g, 6.29 mmol) in dry DMF (1.1 mL) under argon and stirring at room temperature. After 2 h, the mixture was fractionated in *t*-BuOMe : H_2O (2 : 1). The organic phase was washed successively with HCl 2 N and brine and dried over anhydrous Na_2SO_4. Once the solvent was evaporated under low pressure we obtained a crude product (2.14 g), which was dissolved in dry DCM (121 mL) under argon and chilled at –20 °C. After adding DIBAL (1 M in hexane, 16 mL) the solution was stirred for 20 min and then, H_2O (49 mL) was added. The mixture was stirred at room temperature for 20 min and filtered through a layer of silica gel/anhydrous Na_2SO_4 (2/1, w/w). The layer was washed with Et_2O and the organic phase was evaporated under low pressure to afford a crude product, which was chromatographed over silica gel column (petroleum ether (bp 30–40 °C)/Et_2O, 85/15, v/v) to obtain **29** (1.7 g, 95%).

(2*E*,4*E*)-6-Triisopropylsilyloxy-5-methyl-2,4-hexadien-1-ol (29). Colourless syrup. IR ν_{max}(film)/cm^{-1} 3327, 2942, 2865, 1641, 1462, 1385, 1113, 1085, 1065, 995 and 882; δ_H(500 MHz; $CDCl_3$; Me_4Si) 6.50 (1H, dd, *J* 11.2 and 15.1, H-3), 6.15 (1H, d, *J* 11.2, H-4), 5.80 (1H, dt, *J* 6.0 and 15.1, H-2), 4.20 (2H, d, *J* 6.0, H-1), 4.14 (2H, s, H-6), 1.72 (3H, s, H-7) and 1.04–1.18 (21H, m, TIPS); δ_C(125 MHz; $CDCl_3$; Me_4Si) 138.3 (C, C-5), 130.8 (CH, C-3), 127.7 (CH, C-2), 122.2 (CH, C-4), 67.9 (CH_2, C-6), 63.8 (CH_2, C-1), 18.1 ($6CH_3$, TIPS), 13.9 (CH_3, C-7) and 12.1 (3CH, TIPS); *m*/*z* (HRMS(FAB)) 307.2071 (M + Na. $C_{16}H_{32}O_2Na$ requires 307.2070).

Preparation of hydroxy-aldehyde 20. The Dess–Martin reagent (1.01 g) was added to a solution of **29** (800 mg, 2.82 mmol) in dry DCM (31 mL) at room temperature under argon. The mixture was left for 50 min under stirring. A saturated solution of $Na_2S_2O_3$ and $NaHCO_3$ was then added dropwise to the mixture and extracted with Et_2O. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, and filtered. The residue obtained after evaporating the solvent under low pressure was dissolved in petroleum ether (bp 30–40 °C)/Et_2O (3/1, v/v) and

filtered through silica gel. The residue (713 mg) obtained after evaporating the solvent under low pressure was dissolved in dry THF (37 mL) and mixed with TBAF (1 M in THF, 16 mL), stirred at room temperature for 65 min, diluted with Et_2O (15 mL), and washed with brine. The organic phase was dried over anhydrous Na_2SO_4 and filtered. The residue obtained after removing the solvent under low pressure was chromatographed in a silica gel column (petroleum ether (bp 30–40 °C)/Et_2O, 35/65, v/v) to obtain **20** (234 mg, 66%).

Preparation of (2*E*,4*E*)-6-hydroxy-2-methyl-2,4-hexadienal (21)

Preparation of hydroxy-acetate 30. A mixture of **29** (730 mg, 2.57 mmol), dry pyridine (11 mL) and acetic anhydride (0.5 mL) was left at room temperature for 2 h and then was worked up as usual to give a crude product (835 mg), which was dissolved in dry THF (33 mL), mixed with TBAF (1 M in THF, 2.2 mL), stirred at room temperature under argon for 15 min, diluted with Et_2O (15 mL), and washed with brine. The organic phase was dried over anhydrous Na_2SO_4 and filtered. The residue obtained after removing the solvent under low pressure was chromatographed in a silica gel column (petroleum ether (bp 30–40 °C)/Et_2O, 1/1, v/v) to obtain **30** (340 mg, 78%).

(2*E*,4*E*)-6-Hydroxy-5-methyl-2,4-hexadienyl acetate (30). Colourless syrup. IR ν_{max}(film)/cm^{-1} 3420, 2917, 2861, 1737, 1662, 1445, 1380, 1365, 1235, 1113, 1071, 1023 and 971; δ_H(400 MHz; $CDCl_3$; Me_4Si) 6.51 (1H, dd, *J* 11.0 and 15.2, H-3), 6.05 (1H, d, *J* 11.0, H-4), 5.71 (1H, dt, *J* 6.3, 15.2, H-2), 4.58 (2H, d, *J* 6.3, H-1), 4.03 (2H, s, H-6), 2.06 (1H, br s, OH), 2.03 (3H, s, $COCH_3$) and 1.75 (3H, s, H-7); δ_C(100 MHz; $CDCl_3$; Me_4Si) 171.9 (C, *C*OCH_3), 140.0 (C, C-5), 131.0 (CH, C-3), 126.8 (CH, C-2), 123.9 (CH, C-4), 68.3 (CH_2, C-6), 65.4 (CH_2, C-1), 21.1 (CH_3, CO*C*H_3) and 14.2 (CH_3, C-7); *m*/*z* (HRMS(FAB)) 193.0838 (M + Na. $C_9H_{14}O_3Na$ requires 193.0841).

Preparation of hydroxy-aldehyde 21. The Dess–Martin reagent (1.05 g) was added to a solution of **30** (340 mg, 2.00 mmol) in dry DCM (11 mL) at room temperature under argon. The mixture was left for 15 min under stirring. A saturated solution of $Na_2S_2O_3$ and $NaHCO_3$ was then added dropwise to the mixture and extracted with Et_2O. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, and filtered. The residue obtained after evaporating the solvent under low pressure was dissolved in petroleum ether (bp 30–40 °C)/Et_2O (3/1, v/v) and filtered through silica gel. The residue (245 mg) obtained after evaporating the solvent under low pressure was dissolved in EtOH (7.60 mL) and NaOH 1 M (3.80 mL) was added dropwise at 0 °C. The mixture was then left at room temperature for 3 h. The solution was neutralized with HCl 1 N (3.80 mL) and the solvent was evaporated under low pressure. The residue was extracted with EtOAc and the solvent was evaporated under low pressure to obtain **21** (151 mg, 60%).

Preparation of (2*E*,4*E*)-2-methyl-2,4-hexadiene-1,6-diol (22)

DIBAL (1 M in hexane, 2 mL) was added to a cold (–20 °C) solution of **30** (100 mg, 0.78 mmol) in dry CH_2Cl_2 (9 mL) under argon with stirring. After 20 min, water (3 mL) was added to the mixture, and it was stirred at room temperature for 20 min. The mixture was filtered through a layer of silica gel/anhydrous Na_2SO_4 (2/1, w/w). The layer was washed with Et_2O and the organic phase was evaporated under low pressure to afford a crude product, which was chromatographed over silica gel column (petroleum ether (bp 30–40 °C)/Et_2O, 35/65, v/v) to obtain **22** (65 mg, 86%).

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