Pergamon

0031-9422(94)00654-7

Phytochemistry, Vol. 38, No. 2, pp. 419–422, 1995

0031-9422/95 $9.50 + 0.00

A NEW HYDROXYTETRADECATRIENOIC ACID AND ITS GLYCERYL ESTERS FROM *VALSA AMBIENS*

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(*Received in revised form* 16 *June* 1994)

Key Word Index—*Valsa ambiens*; Ascomycetes; hydroxytetradecatrienoic acid; 1-hydroxytetradecatrienoyl-*sn*-glycerol; 3-hydroxytetradecatrienoyl-*sn*-glycerol; growth inhibition.

Abstract—A new hydroxytetradecatrienoic acid and its two glyceryl esters were isolated from a culture filtrate of *Valsa ambiens*. 1-Hydroxytetradecatrienoyl-*sn*-glycerol and 3-hydroxytetradecatrienoyl-*sn*-glycerol, only differing in the configuration of carbon 2′, were separated as their corresponding MTPA esters. The absolute stereochemistry of the asymmetric centre of the acid and the two asymmetric centres of the esters were determined by using advanced Mosher methodology. The glyceryl esters exhibited an inhibitory effect on the growth of lettuce roots and hypocotyls.

INTRODUCTION

Valsa ambiens is an Ascomycete fungal pathogen causing *Valsa* canker of cherry (*Prunus sargentii*). We reported two phenolic compounds (**1** and **2**) from *V. ambiens* [1] having an inhibitory effect on the growth of lettuce roots and hypocotyls. Continuing our investigations on the bioactive products amongst the metabolites, a new hydroxytetradecatrienoic acid (**3**) and its glyceryl esters (**4a** and **4b**) were isolated from a culture filtrate of this fungus. This paper reports the isolation, structural determination, stereochemistry and bioactivity of the new compounds (**3**, **4a** and **4b**).

RESULTS AND DISCUSSION

The ethyl acetate extract of the culture filtrate inhibited the growth of lettuce roots and hypocotyls. Purification was done by column chromatography on silica gel and bioactive fractions only were retained for structural elucidation. Finally, **3** and **4** (mixture of **4a** and **4b**) were isolated from these active fractions.

Compound **3** was obtained as a light yellow oil and responded positively to Bromocresol Green reagent for acid compounds. The IR spectrum showed a broad absorption band at 3320 cm^{-1} due to a hydroxyl group and an absorption band at 1680 cm^{-1} due to an acid carboxyl group. The FD mass spectrum of **3** showed a $[M + H]^+$ at *m/z* 239. The $[M]^+$ did not appear in its EI mass spectrum, but the peak corresponding to its dehydration appeared at *m/z* 220. The molecular formula of **3** was determined as $C_{14}H_{22}O_3$ from the HREI mass spectrum of *m/z* 220 ($C_{14}H_{20}O_2$). The ^{13}C NMR and DEPT experiments revealed that **3** contained 14 carbons: one methyl (δ23.5), five methylenes (δ26.8, 33.0, 33.6, 34.1, 39.6), six olefinic (δ124.2, 130.4, 130.5, 132.2, 143.0, 144.2), a methine bearing an oxygen (δ68.4) and a carbonyl group (δ170.0). Assignments were made in conjunction with ^1H NMR and ^1H–^{13}C COSY spectra. The ^1H–^1H COSY NMR spectrum was well-resolved. Starting from the H-2 signal at δ5.78, every proton on the 14 carbons was assigned unambiguously and the chemical structure was determined to be 13-hydroxytetradeca-2,3,8-trienoic acid. Two *E*-double bonds at C-2–C-3 and C-4–C-5 were directly revealed as ethylene groups by the ^1H NMR coupling constant (15.5 and 15.2 Hz), but the double bond at C-8–C-9 could not be determined directly from ^1H NMR signals because the two chemical shifts were very close. It was deduced as *E* from ^1H NMR spin decoupling spectra by irradiation of H-7 (δ2.15) and H-10 (δ2.0) showing the coupling constant (15.3 Hz) between H-8 and H-9.

The absolute configuration at C-13 was determined by an advanced Mosher method [2, 3], based on analyses of the difference of the chemical shifts between the (+)- and (−)-MTPA (α-methoxytrifluoromethylphenylacetyl) esters of the alcohols. In this process, **3** was methylated with diazomethane and converted to the corresponding (*S*)-(−)- and (*R*)-(+)-13-*O*-MTPA esters (**3a-1**, **3a-2**), respectively. The chemical shifts of the methylene protons at C-12 and C-11 neighbouring the chiral C-13 could not be recognized due to overlap with each other. But the chemical shifts of methyl groups (C-14) of the (*S*)-(−)-ester (**3a-1**) and the (*R*)-(+)-ester (**3a-2**) were δ1.26 and 1.34, respectively (Fig. 1). The difference of the chemical shift was − 0.08 [$\Delta\delta = \delta(-) - \delta(+)$] which

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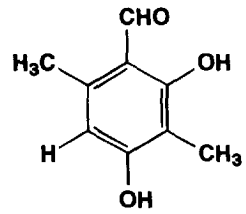

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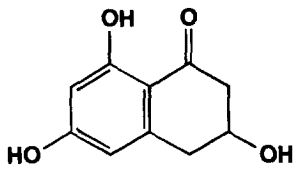

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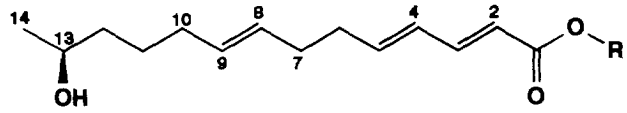
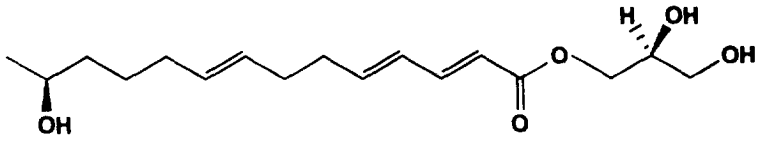
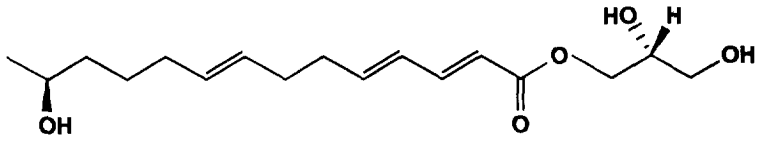


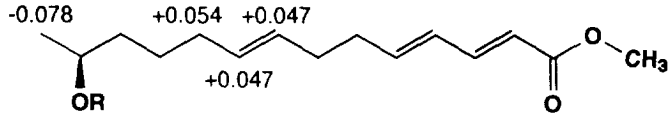
3: R=H
3a: R=CH3
4: R=$CH_2CHOHCH_2OH$



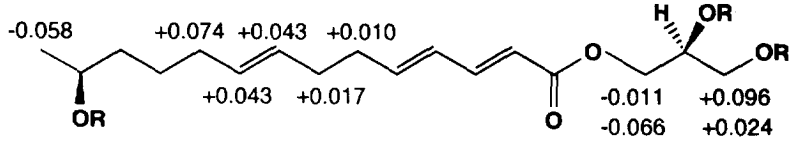
4a



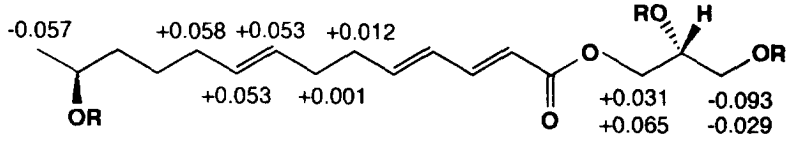
4b



3a-1: R = S(-) MTPA
3a-2: R = R(+) MTPA



4a-1: R= S(-) MTPA
4a-2: R= R(+) MTPA



4b-1: R=S(-)MTPA
4b-2: R=R(+)MTPA

Fig. 1. Difference between [*S*(−)MTPA − R(+)MTPA] ^{1}H NMR chemical shifts of MTPA esters of **3a**, **4a** and **4b**.

Table 1. ^{13}C NMR chemical shifts of **3** and **4** (125 MHz, CD_3OD)

C	3	4
1	170.0	168.9
2	124.2	119.8
3	144.2	147.0
4	130.4	129.9
5	143.0	145.7
6	34.1	34.1
7	33.0	32.8
8	130.5	130.4
9	132.2	132.4
10	33.6	33.6
11	26.8	26.8
12	39.6	39.6
13	68.4	68.4
14	23.5	23.5
1′	—	66.5
2′	—	71.2
3′	—	64.1

Table 2. ^1H NMR spectral data of **3** and **4** (500 MHz, CD_3OD, TMS as int. standard)

C	3	4
2	5.78 *d* (15.5)	5.85 *d* (15.4)
3	7.21 *dd* (10.2, 15.5)	7.29 *dd* (10.4, 15.4)
4	6.24 *dd* (10.2, 15.2)	6.26 *dd* (15.2, 10.4)
5	6.14 *dt* (5.5, 15.2)	6.19 *dt* (6.6, 15.2)
6	2.25 *m*	2.25 *m*
7	2.15 *m*	2.14 *m*
8	5.42 *dt* (5.7, 15.3)	5.41 *dt* (5.4, 15.3)
9	5.46 *dt* (5.9, 15.3)	5.46 *dt* (5.8, 15.3)
10	2.00 *m*	2.00 *m*
11	1.41 *m*	1.48 *m*
12	1.39 *m*	1.40 *m*
13	3.70 *m*	3.70 *m*
14	1.13 *d* (5.9)	1.12 *d* (6.2)
1′-a	—	4.11 *dd* (11.4, 4.3)
1′-b	—	4.21 *dd* (11.4, 6.3)
2′	—	3.84 *m*
3′-a	—	3.55 *dd* (11.3, 5.8)
3′-b	—	3.57 *dd* (11.3, 5.4)

indicated an *S*-configuration at C-13 for **3**. This was supported by the positive numbers of the difference of H-8–H-10 between **3a-1** and **3a-2**. Thus, the structure of **3** was deduced as 13-*S*-hydroxy-2*E*, 3*E*, 8*E*-tetradecatrienoic acid.

Compound **4** was obtained as a light yellow viscous liquid. The infrared spectrum indicated the presence of hydroxyl (3325 cm^{-1}) and ester (1710 cm^{-1}) moieties. The FD mass spectrum of **4** showed a $[M + H]^+$ at *m/z* 313. The EI mass spectrum showed no $[M]^+$, but fragment ions at *m/z* 263, resulting from the loss of one water and two water and a CH fragment, respectively. The molecular formula was determined as $C_{17}H_{28}O_5$ from HREI mass spectral measurement of *m/z* 263 $[M - 2H_2O - CH]^+$, ($C_{16}H_{23}O_3$). In comparison with the ^1H NMR spectra of **3**, the ^1H NMR signals of the partial structure of **4** were in good agreement with those of **3**. The other proton signals at δ3.56 (1H, *m*), 3.84 (2H, *m*), 4.18 (2H, *m*) correlated with the carbon signals at δ64.1 (CH_2), 66.5 (CH), 71.2 (CH_2), indicating esters of glycerols.

In order to determine the absolute configuration, **4** was converted to the corresponding (*S*)-(−)-MTPA ester (**4a-1**, **4b-1**) and (*R*)-(+)-MTPA ester (**4a-2**, **4b-2**). The (*S*)-(−)-MTPA ester showed two peaks on HPLC which were separated into two compounds **4a-1** and **4b-1**. The (*R*)-(+)-MTPA esters (**4a-2**, **4b-2**) of **4** were separated in a similar manner. Thus, **4** was a mixture of **4a** and **4b**, showing that the configuration at C-13 for both is *S* by means of the advanced Mosher methodology (Fig. 1). A difference of chemical shifts in the glycerol moiety of the two compounds (**4a**, **4b**) was apparent and the absolute configurations at C′-2 were determined to be *S* in **4a** and *R* in **4b**. Thus, the structure of **4a** was deduced to be 1-(13-*S*-hydroxy-2*E*, 3*E*, 8*E*-tetradecatrienoyl)-*sn*-glycerol, and that of **4b**, 3-(13-*S*-hydroxyl-2*E*, 3*E*, 8*E*-tetradecatrienoyl)-*sn*-glycerol.

Compounds **3** and **4** were found to be active in inhibiting the growth of roots and hypocotyls of germinating lettuce. The growth inhibitory ratios of roots and hypocotyls were 25.6%, 46.6% for **3**, and 33.4%, 63.5% for **4** at 1 mM, respectively.

EXPERIMENTAL

NMR spectra were measured in solns of CD_3OD or $CDCl_3$. CC was done using Merck Kieselgel 60 (0.04–0.063 mm). HPLC was performed on columns of Inertsil ODS-2 (GL Science, 4.6 × 250 mm) and Wakosil 5sil-120 [Wako. 6.0 × 250 mm (w)].

The fungus was cultured in a potato–glucose medium in stationary flasks for 35 days at 25°; the culture filtrate was concd *in vacuo* to 1/5th its vol. The concd culture filtrate was extracted with EtOAc. The extract was evapd to dryness *in vacuo* and the residue chromatographed on a silica gel column successively eluted with $CHCl_3$, MeOH–$CHCl_3$ (1:19), MeOH–$CHCl_3$ (1:4) and MeOH. After separating the eluate by silica gel CC with MeOH–$CHCl_3$ (1:19), 2 active frs A and B were obtained from the later fr. Compounds **3** and **4** were isolated by prep. TLC on silica gel with MeOH–$CHCl_3$ (1:5) and a Lobar Lichroprep RP-8 column with MeOH–H_2O (17:3).

Compound **3**. $[\alpha]_D^{23}$ + 1.24 (MeOH; *c* 0.3). IR ν_{max} cm^{-1}: 3320, 1680. HREIMS: $C_{14}H_{20}O_2$ $[M - H_2O]^+$, (calcd 220.1463; found 220.1464); EIMS *m/z* (rel. int.): 220 (1.2), 202 (1.7), 112 (21.2), 109 (23.2), 93 (10.5), 91 (15.1), 79 (19.7), 67 (100); FDMS *m/z* 239 $[M + H]^+$.

Compound **3a**. Compound **3** (4 mg) was dissolved in Et_2O–CH_2N_2 (1 ml) and stirred for 2 hr. After evaporating the reaction soln, the Me ester **3a** (3.5 mg) was obtained by prep. TLC on silica gel with MeOH–$CHCl_3$ (1:20). FDMS *m/z* (rel. int): 253 $[M + H]^+$ (100).

^{1}H NMR (500 MHz, $CDCl_3$): δ1.13 (3H, *d*, *J* = 6 Hz, H-14), 1.40 (4H, *m*, H-11, H-12), 2.00 (2H, *m*, H-10), 2.13 (2H, *m*, H-7), 2.21 (2H, *m*, H-6), 3.68 (1H, *m*, H-13), 3.71 (3H, *s*, H-15), 5.40 (2H, *m*, H-8, H-9), 5.78 (1H, *d*, *J* = 15.3 Hz, H-2), 6.17 (2H, *m*, H-4, H-5), 7.23 (1H, *m*, H-3).

Compounds **3a-1** *and* **3a-2**. DMAP (3.19 mg, 11.9 μmol), pyridine (1 drop), and MTPACl (1 drop) [(*S*)-(−)-MTPACl for **3a-1** and (*R*)-(+)-MTPACl for **3a-2**] were added to a stirred soln of **3a** (1.5 mg, 5.95 μmol) in CH_2Cl_2 (0.5 ml). After 24 hr, the reaction mixt. was concd under red. pres. The residue was subjected to prep. TLC on silica gel using hexane–EtOAc (2:1) to give the (*S*)-(−)-MTPA ester **3a-1** and the (*R*)-(+)-MTPA ester **3a-2**, respectively. Compounds **3a-1** and **3a-2** were purified by HPLC on a Wakopak column with hexane–EtOAc (4:1). Compound **3a-1** FDMS *m/z*: 468 $[M]^+$, 469 $[MH]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ1.26 (3H, *d*, *J* = 6 Hz, H-14), 1.57 (4H, *m*, H-11, H-12), 2.0 (2H, *m*, H-10), 2.16 (2H, *m*, H-7), 2.21 (2H, *m*, H-6), 3.54 (3H, *s*, H-15), 5.14 (1H, *m*, H-13), 5.38 (2H, *m*, H-8, H-9), 5.80 (1H, *d*, *J* = 15.3 Hz), 6.17 (2H, *m*, H-4, H-5), 7.26 (1H, *m*, H-3). Compound **3a-2** FDMS *m/z*: 468 $[M]^+$, 469 $[MH]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ1.34 (3H, *d*, *J* = 6 Hz, H-14), 1.56 (4H, *m*, H-11, H-12), 2.0 (2H, *m*, H-10), 2.15 (2H, *m*, H-7), 2.21 (2H, *m*, H-6), 3.57 (3H, *s*, H-15), 5.16 (1H, *m*, H-13), 5.32 (2H, *m*, H-8, H-9), 5.80 (1H, *d*, *J* = 15.3 Hz, H-8, H-9), 6.16 (2H, *m*, H-4, H-5), 7.26 (1H, *m*, H-3).

Compound **4**. $[\alpha]_D^{23}$ + 1.64° (MeOH; *c* 0.6). IR ν_{max} cm^{-1}: 3325, 1710. HREIMS *m/z*: 263 $[M - 2 \times H_2O - CH]^+$, $C_{16}H_{23}O_3$ (calcd 263.1647, found 263. 1675); EIMS *m/z* (rel. int.): 263 (0.6), 220 (3.3), 186 (9.2), 112 (6.7), 109 (23.7), 94 (100), 67 (94.4); FDMS *m/z*: 313 $[MH]^+$.

Compounds **4a-1**, **4a-2**, **4b-1**, **4b-2**. To a stirred soln of **4** (2 mg, 6.14 μmol) in CH_2Cl_2 (0.5 ml) were added DMAP (3.29 mg, 12.2 μmol), pyridine (2 drops), MTPACl (1 drop) [(*S*)-(−)-MTPACl for **4a-1** and **4b-1**, (*R*)-(+)-MTPACl for **4a-2** and **4b-2**]. After 48 hr, the reaction mixt. was concd under red. pres. The residue was subjected to prep. TLC on silica gel with hexane–EtOAc (2:1) to give the *tris*-MTPA esters. The (*S*)-(−)-MTPA esters **4a-1** and **4b-1** were sepd by HPLC on a Wakopak column with hexane–EtOAc (4:1); (*R*)-(+)-MTPA esters **4a-2** and **4b-2** were sepd in the same way. Compound **4a-1** FDMS *m/z*: 960 $[M]^+$, 961 $[MH]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ1.27 (3H, *d*, *J* = 6.0 Hz, H-14), 1.34 (4H, *m*, H-11, H-12), 2.03 (2H, *m*, H-10), 2.16 (2H, *m*, H-7), 2.25 (2H, *m*, H-6), 5.16 (1H, *m*, H-13), 5.40 (2H, *m*, H-8, H-9), 5.75 (1H, *d*, *J* = 15.3 Hz, H-2), 6.17 (2H, *m*, H-4, H-5), 7.23 (1H, *dd J* = 15.3 Hz, H-3), 4.26 (1H, *m*, H-3′), 4.43 (1H, *m*, H-3′), 4.39 (1H, *m*, H-1′), 4.66 (1H, *m*, H-1′), 5.62 (1H, *m*, H-2′). Compound **4a-2**: FDMS *m/z*: 960 $[M]^+$, 961 $[MH]^+$. ^{1}H NMR (500 MHz, $CHCl_3$): δ1.32 (3H, *d*, *J* = 6.0 Hz, H-14), 1.32 (4H, *m*, H-11, H-12), 1.95 (2H, *m*, H-10), 2.14 (2H, *m*, H-7), 2.24 (2H, *m*, H-6), 5.15 (1H, *m*, H-13), 5.36 (2H, *m*, H-8, H-9), 5.70 (1H, *d*, *J* = 15.3 Hz, H-2), 6.16 (2H, *m*, H-4, H-5), 7.20 (1H, *dd*, *J* = 15.3 Hz, H-3), 4.17 (1H, *m*, H-3′), 4.36 (1H, *m*, H-3′), 4.44 (1H, *m*, H-1′), 4.73 (1H, *m*, H-1′), 5.57 (1H, *m*, H-2′). Compound **4b-1** FDMS *m/z*: 960 $[M]^+$, 961 $[MH]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ1.27 (3H, *d*, *J* = 6.0 Hz, H-14), 1.34 (4H, *m*, H-11, H-12), 2.0 (2H, *m*, H-10), 2.16 (2H, *m*, H-7), 2.26 (2H, *m*, H-6), 5.16 (1H, *m*, H-13), 5.41 (2H, *m*, H-8, H-9), 5.71 (1H, *d*, *J* = 15.3 Hz, H-2), 6.17 (2H, *m*, H-4, H-5), 7.25 (1H, *dd*, *J* = 15.3 Hz, H-3), 4.17 (1H, *m*, H-3′), 4.36 (1H, *m*, H-3′), 4.44 (1H, *m*, H-1′), 4.73 (1H, *m*, H-1′), 5.57 (1H, *m*, H-2′). Compound **4b-2**. FDMS *m/z*: 960 $[M]^+$, 961 $[MH]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ1.32 (3H, *d*, *J* = 6.0 Hz, H-14), 1.34 (4H, *m*, H-11, H-12), 1.95 (2H, *m*, H-10), 2.16 (2H, *m*, H-7), 2.24 (2H, *m*, H-6), 5.16 (1H, *m*, H-13), 5.35 (1H, *m*, H-8, H-9), 5.75 (1H, *d*, *J* = 15.3 Hz, H-2), 6.16 (2H, *m*, H-4, H-5), 7.26 (1H, *dd*, *J* = 15.3 Hz, H-3), 4.27 (1H, *m*, H-3′), 4.39 (1H, *m*, H-3′), 4.41 (1H, *m*, H-1′), 4.67 (1H, *m*, H-1′), 5.62 (1H, *m*, H-2′).

Acknowledgements—We thank Mr K. Watanabe and Ms E. Fukushi of this University for MS measurements.

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