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A HYDROXYTETRADECATRIENOIC ACID FROM *MYCOSPHAERELLA RUBELLA*

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Key Word Index—*Mycosphaerella rubella*; Ascomycetes; dihydroxytetradecatrienoic acids; structural elucidation; absolute configuration.

Abstract—Investigations on the acidic fractions from extracts of a culture of *Mycosphaerella rubella* led to the isolation of a new unsaturated dihydroxy acid, 6,13-dihydroxytetradeca-2,4,8-trienoic acid, and two of its derivatives, which are probably artifacts. Their structures were determined on the basis of ^{1}H and ^{13}C NMR evidence. The absolute configuration of the chiral centres of the new acid was elucidated using the modified Mosher's method. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The fungal genus *Mycosphaerella* includes many phytopathogenic species which are sources of interesting metabolites, such as: epoxydon, rosigenin, mycochromone and dothistromin [1]. Some of these compounds are phytotoxic and also exhibit pharmacological activity. Previously [2, 3], we reported the isolation from *M. rubella* (the causal agent of a disease of *Angelica silvestris*) of rubellins A–D, novel anthraquinone metabolites which, from a biogenetic point of view, may be considered to be dimers of the anthraquinone, chrysophanol. From the ethyl acetate extracts of the same fungus, after concentration, a less soluble compound was separated and identified as 9,10-phenanthrenequinone, previously isolated from the lichen, *Parmelia birulae*, and named, biruloquinone, the revised structure was published by us [4]. Recently, the isolation of the Ascomycete from *Valsa ambiens*, of a new hydroxytetradecatrienoic acid growth inhibitor has been reported [5] and other hydroxylated unsaturated fatty acids with antiviral activity from the Basidiomycete, *Filoboletus*, have been described [6].

In the present paper, we describe the isolation, structural determination and assignment of the absolute configuration at C-6 and -13 of a new unsaturated hydroxyacid (**1**) from *M. rubella*.

RESULTS AND DISCUSSION

The acidic fractions of the ethyl acetate extracts of the fungus were purified by column chromatography on silica gel and prep. TLC (see Experimental), and compounds **1**, **3** and **5** were isolated. The IR spectrum of **1** exhibited absorptions at 3400 and 1695 cm^{-1} indicating the presence of hydroxyl and unsaturated carboxylic groups. Reaction of **1** with CH_2N_2 afforded the expected methyl ester **2**, the molecular formula of which was established as $C_{15}H_{26}O_4$ by mass spectrometry. Its ^{13}C NMR spectrum showed the presence of seven sp^2 carbons, participating in the carboxylic group and in three disubstituted carbon–carbon bonds, and eight resonances due to two oxygen-bearing methines, four methylenes and two methyl sp^3 carbon atoms. Chemical shift criteria, as corroborated by ^{1}H-^{1}H decoupling experiments and coupling constant analysis, indicated that the olefinic double bonds (all *E* as evidenced by ^3J = 15.3–15.5 Hz) are contained in a —C(11)H$_2$—C(10)H$_2$—C(9)H=C(8)H—C(7)H$_2$—C(6)HOH—C(5)H=C(4)H—C(3)H=C(2)H—C(1)O$_2$Me partial structure, while the remaining protons are part of a C(14)H$_3$—C(13)HOH—C(12)H$_2$—-moiety.

At this stage, it was sufficient to link C-11 to C-12 to obtain the gross structure of **2**, the only problem to be solved being the assignment of the absolute configurations at C-6 and C-13. For this purpose, we applied the modified Mosher's method [7] to the (*S*)- and (*R*)-MTPA (α-methoxy-α-trifluoromethyl) phenylacetic acid diesters **7** and **8** obtained by reacting **2** with the corresponding (−) and (+) MTPA acids. The $\Delta\delta$ values (δ_S–δ_R) depicted in Fig. 1 permitted us to assign as *S* and *R* the chiralities at C-6 and C-13, because the protons on the right hand side of the MTPA plane shown in Fig. 2 (i.e., H-2 → H-5 and H$_3$-14, respectively) had positive numbers. The structure

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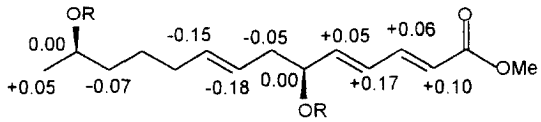
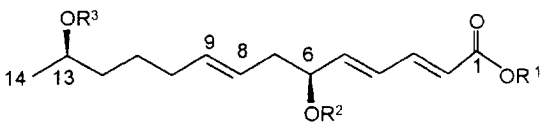


7 R = S (-) MTPA

8 R = R (+) MTPA

Fig. 1. Differences in proton chemical shifts ($\Delta\delta = \delta_S - \delta_R$) of MTPA Mosher ester derivatives of compound **2**.



1 $R^1 = R^2 = R^3 = H$

2 R^1 = Me; $R^2 = R^3$ = H

3 $R^1 = R^2$ = H ; R^3 = Ac

4 R^1 = Me; R^2 = H ; R^3 = Ac

5 $R^1 = R^2$ = H; R^3 = COH

6 R^1 = Me; R^2 = H; R^3 = COH

of compound **1** was therefore determined to be 6-*S*,13-*R*-dihydroxy-2*E*,4*E*,8*E*-tetradecatrienoic acid.

Comparison between the ^{1}H NMR spectrum of **2** with those of the methyl esters **4** and **6** indicated that these compounds share the same basic structure, the only significant difference being the presence of additional three- and one-proton singlets at 2.02 and 8.04 ppm, respectively. The concomitant downfield shifts experienced by H-13 ($\Delta\delta$ = 1.21 and 1.36 ppm, respectively) identified compounds **4** and **6** as the corresponding 13-acetyl and 13-formyl derivatives of **2**.

Compounds **3** and **5** probably are artifacts formed during the purification of the mixture of metabolites. Unfortunately, the lack of material did not enable us to resolve this problem.

Compound **1** showed weak antibacterial activity (100 μg per disc) against *Sarcina lutea*, *Bacillus cereus* and *B. subtilis* but not against *Escherichia coli*; no activity was found against *Saccharomyces cerevisiae*.

EXPERIMENTAL

Flash CC was performed with Merck silica gel (0.04–0.063 mm) and TLC with Merck HF254 silica gel. MS were recorded at 60 eV. ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR at 63 MHz, with TMS as int. standard.

Isolation and purification of acids **1**, **3** and **5**.

A strain of *M. rubella* obtained from Centraalbureau voor Schimmelcultures, Baarn, was cultivated as previously described [2]. The EtOAc extracts were washed with a 10% $NaHCO_3$ soln, the last one was acidified and extracted with more EtOAc. The acidic extract was evapd to dryness *in vacuo* and chromatographed on a silica gel column eluted with CH_2Cl_2 containing increasing amounts of MeOH (from 1 to 10%), and successively by prep. TLC using benzene–Et_2O–HCO_2H (3:1:1) to give pure metabolite **1** and a mixt. of compounds **3** and **5**.

Compound **1**. Oil $[\alpha]_D$ +18° (MeOH; *c* 0.1). IR ν_{max} cm^{-1}: 3400, 1695. ^{1}H NMR ($CDCl_3$): δ 7.35 (1H, *br dd*, *J* = 15.4 and 11.2 Hz, H-3), 6.42 (1H, *br dd*, *J* = 15.4 and 11.2 Hz, H-4), 6.17 (1H, *br dd*, *J* = 15.4 and 5.3 Hz, H-5), 5.89 (1H, *br d*, *J* = 15.4 Hz, H-2), 5.55 (1H, *br dt*, *J* = 15.4 and 6.7 Hz, H-9), 5.39 (1H, *br dt*, *J* = 15.4 and 6.7 Hz, H-8), 5.00 (3H, *br s*, 2 × OH and CO_2H), 4.28 (1H, *m*, H-6), 3.82 (1H, *tq*, *J* = 6.2 and 6.3 Hz, H-13), 2.5–1.9 (4H, *m*, H_2-7 and -10), 1.6–1.3 (4H, *m*, H_2-11 and -12), 1.19 (3H, *t J* = 6.3 Hz, H_3-14).

Compound **2**. Compound **1** (20 mg) was methylated with CH_2N_2. Evapn of the solvent and prep. TLC using hexane–EtOAc (2:1) gave ester **2** (15 mg) as an oil: $[\alpha]_D$ +15.2° (MeOH; *c* 0.2). IR ν_{max} cm^{-1} 3400, 1720. CIMS *m/z*: 269 $[MH]^+$. ^{1}H NMR ($CDCl_3$): δ 7.29 (1H, *br dd*, *J* = 15.5 and 11.0 Hz, H-3), 6.39 (1H, *dddd*, *J* = 15.4, 11.0, 1.4 and 0.8 Hz, H-4), 6.14 (1H, *br dd*, *J* = 15.4 and 5.3 Hz, H-5), 5.90 (1H, *dd*, *J* = 15.5 and 0.8 Hz, H-2), 5.57 (1H, *dtt*, *J* = 15.3, 6.5 and 1.2 Hz, H-9), 5.39 (1H, *dtt*, *J* = 15.3, 7.0 and 1.2 Hz, H-8), 4.26 (1H, *dddd*, *J* = 7.1, 5.5, 5.3 and 1.4 Hz, H-6), 3.68 (1H, *tq*, *J* = 6.3 and 6.2 Hz, H-13), 3.64 (3H, *s*, CO_2Me), 2.32 (1H, *br dddd*, *J* = 14.0, 7.0, 5.5 and 1.2 Hz, H-7a), 2.24 (1H, *br dddd*, *J* = 14.0, 7.1, 7.0 and 1.2 Hz, H-7b), 2.05 (2H, *m*, H_2-10), 1.80 (2H, *brs*, OH-6 and -13), 1.55–1.35 (4H, *m*, H_2-11 and -12), 1.19 (3H, *t*, *J* = 6.2 Hz, H_3-14). ^{13}C NMR ($CDCl_3$): δ 167.5 (*s*, C-1), 144.5 and 144.2 (*d*, C-3 and C-5), 135.2 and 127.3 (*d*, C-8 and C-9), 124.9 and 120.9 (*d*, C-2 and C-4), 70.9 and 67.9 (*d*, C-6 and C-13), 51.6 (*q*, OMe), 40.5 (*t*, C-7), 38.7 (*t*, C-12), 32.5 (*t*, C-10), 25.4 (*t*, C-11), 23.6 (*q*, C-14).

Compounds **7** *and* **8**. To two solns of **2** (10 mg) in CH_2Cl_2 (2 ml) containing DMAP (few crystals) and DCC (30 mg), (*S*)-(−)-MTPA and (*R*)-(+)-MTPA were added, respectively. Each mixt. was stirred at room temp. for 6 h and the products obtained (**7** and **8**) were purified by prep. TLC using hexane–EtOAc (2:1). *Compound* **7**. ^{1}H NMR ($CDCl_3$): δ 7.6–7.3 (10H, *m*, ArH), 7.23 (1H, *br dd*, *J* = 15.4 and 11.1 Hz, H-3), 6.33 (1H, *br dd*, *J* = 15.3 and 11.1 Hz, H-4), 6.01 (1H, *br dd*, *J* = 15.3 and 6.7 Hz, H-5), 5.91 (1H, *br d*, *J* = 15.4 Hz, H-2), 5.55 (1H, *m*, H-6), 5.35 (1H, *br dt*, *J* = 15.4 and 6.7 Hz, H-9), 5.14 (1H, *br dt*, *J* = 15.4 and 6.9 Hz, H-8), 5.13 (1H, *ddq*, *J* = 7.0, 5.5 and 6.3 Hz, H-13), 3.90 (3H, *s*, CO_2Me), 3.56 (6H, *q*, *J* = 1.2

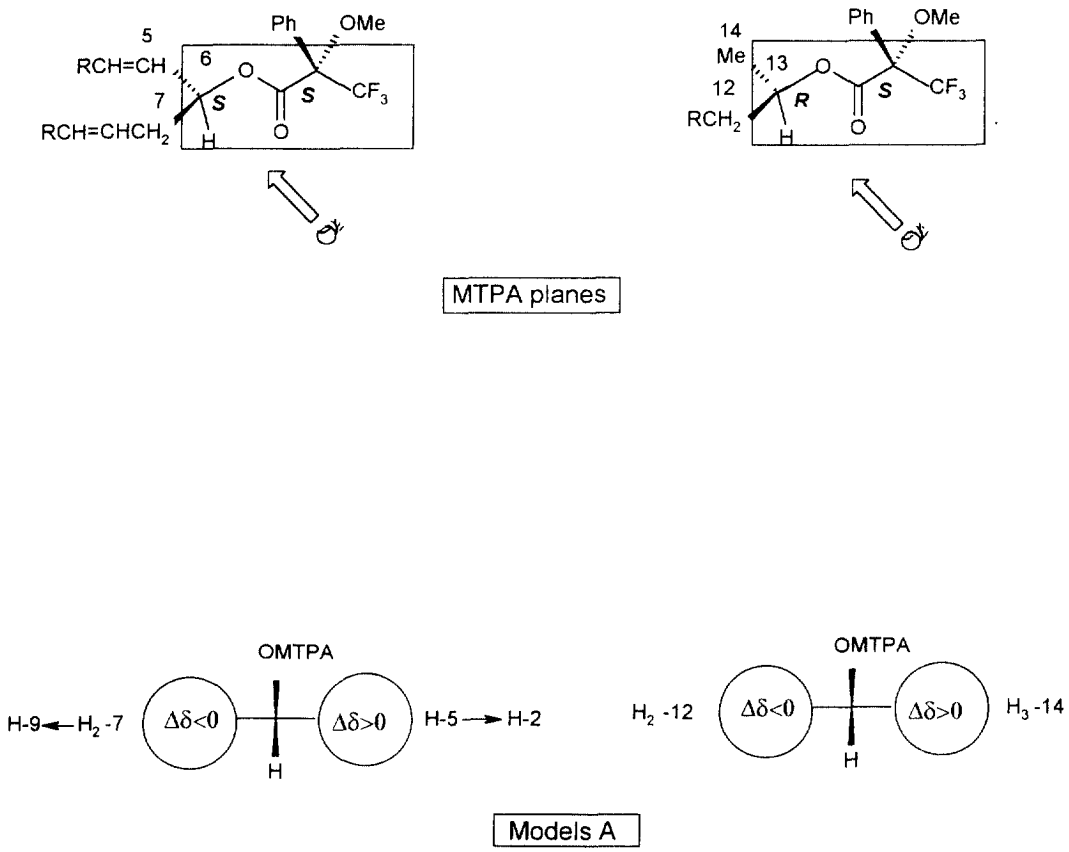


Fig. 2. MTPA planes and models A for the (*S*)-MTPA diester **7** used to assign the absolute configurations at C-6 and C-13.

Hz, 2 × OMe), 2.38 (2H, *m*, H_2-7), 1.87 (2H, *m*, H_2-10), 1.7–1.4 (4H, *m*, H_2-11 and -12), 1.32 (3H, *d*, J = 6.3 Hz, H_3-14). *Compound* **8**. ^{1}H NMR ($CDCl_3$): δ 7.6–7.3 (10H, *m*, ArH), 7.17 (1H, *br dd*, J = 15.4 and 11.0 Hz, H-3), 6.16 (1H, *br dd*, J = 15.4 and 11.0 Hz, H-4), 5.94 (1H, *br dd*, J = 15.4 and 6.5 Hz, H-5), 5.81 (1H, *br d*, J = 15.4 Hz, H-2), 5.56 (1H, *m*, H-6), 5.50 (1H, *br dt*, J = 15.4 and 6.7 Hz, H-9), 5.32 (1H, *br dt*, J = 15.4 and 6.7 Hz, H-8), 5.12 (1H, *ddq*, J = 7.0, 5.3 and 6.3 Hz, H-13), 3.75 (3H, *s*, CO_2Me), 3.56 and 3.54 (6H, *q*, J = 1.2 Hz, 2 × OMe), 2.43 (2H, *m*, H_2-7), 2.00 (2H, *m*, H_2-10), 1.8–1.5 (4H, *m*, H_2-11 and -12), 1.27 (3H, *d*, J = 6.3 Hz, H_3-14).

Compounds **3** *and* **5**. The mixt. was analysed by NMR. *Compound* **3**. ^{1}H NMR ($CDCl_3$): δ 7.34 (1H, *br dd*, J = 15.3 and 11.2 Hz, H-3), 6.41 (1H, *br dd*, J = 15.4 and 11.2 Hz, H-4), 6.17 (1H, *br dd*, J = 15.4 and 5.2 Hz, H-5), 5.89 (1H, *br d*, J = 15.3 Hz, H-2), 5.55 (1H, *br dt*, J = 15.4 and 6.7 Hz, H-9), 5.39 (1H, *br dt*, J = 15.4 and 6.7 Hz, H-8), 5.20 (2H, *brs*, OH and CO_2H), 4.89 (1H, *m*, H-13), 4.28 (1H, *m*, H-6), 2.5–2.1 (4H, *m*, H_2-7 and -10), 2.04 (3H, *s*, 13-OAc), 1.7–1.3 (4H, *m*, H_2-11 and -12), 1.21 (3H, *d*, J = 6.2 Hz, H_3-14). *Compound* **5**. ^{1}H NMR ($CDCl_3$): δ 8.04 (1H, *s*, 13-CHO), 5.03 (1H, *m*, H-13); the remaining signals being similar to those exhibited by **3**.

Compounds **4** *and* **6**. The mixt. was methylated with CH_2N_2 and the Me esters separated by prep. TLC on silica gel using hexane–EtOAc (4:1). *Compound* **4**. Oil. CIMS *m/z*: 311 $[MH]^+$. ^{1}H NMR ($CDCl_3$): δ 7.28 (1H, *br dd*, J = 15.5 and 11.1 Hz, H-3), 6.39 (1H, *br dd*, J = 15.4 and 11.1 Hz, H-4); 6.13 (1H, *br dd*, J = 15.4 and 5.2 Hz, H-5), 5.90 (1H, *br d*, J = 15.5 Hz, H-2), 5.56 (1H, *br dt*, J = 15.3 and 6.7 Hz, H-9), 5.40 (1H, *br dt*, J = 15.3 and 6.7 Hz, H-8), 4.89 (1H, *m*, H-13), 4.27 (1H, *m*, H-6), 3.75 (3H, *s*, 1-OMe), 2.5–2.0 (4H, *m*, H_2-7 and -10), 2.02 (3H, *s*, 13-OAc), 1.7–1.3 (4H, *m*, H_2-11 and -12), 1.20 (3H, *d*, J = 6.2, H_3-14). *Compound* **6**. Oil. CIMS, *m/z*: 297 $[MH]^+$. ^{1}H NMR ($CDCl_3$): δ 8.04 (1H, *s*, 13-OCHO), 5.04 (1H, *m*, H-13); the remaining signals being similar to those exhibited by **4**.

Biological tests

Antibacterial and antifungal activity were tested with paper discs (6 mm diam.), soaked with metabolite **1** (100 and 50 μg dissolved in $CHCl_3$–MeOH, 2:1) and placed in a suitable culture medium on Petri dishes with *Sarcina lutea* (DMS 348), *Escherichia coli* (IPV 287), *Bacillus cereus* (ATCC 10702), *Bacillus subtilis* (ATCC 6633) and *Saccharomyces cerevisiae* (NCYC 729) as test micro-organisms.

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