Bárbara Gigante[1]
Ana M. Silva[1]
Maria João Marcelo-Curto[1]
Sónia Savluschinske Feio[1]
José Roseiro[1]
Lucinda V. Reis[2]

Structural Effects on the Bioactivity of Dehydroabietic Acid Derivatives

Abstract

The synthesis and the evaluation of the antimicrobial activity against a filamentous fungus, yeasts and bacteria of 15 hydrophenanthrene compounds derived from dehydroabietic acid, bearing different functional groups and different stereochemistry of the A/B ring junction are disclosed. The results obtained showed how their activity is dependent of the functionality at C-18, which can be increased by deisopropylation or introduction of other groups into the molecule. While the filamentous fungus tested is sensitive to almost all of the compounds under study, the aldehyde function showed to be of major importance to the inhibition of yeast. Alcohols and aldehyde C-18 derivatives also inhibit the growth of a Gram-positive bacteria, whereas Gram-negative are not sensitive.

Key words
Bioactivity · diterpenes · abietic acid · dehydroabietic acid · antimicrobial activity

Introduction

In recent years, many pathogenic microorganisms have assumed a serious role, either in human or animal health, due to their resistance to the known chemical control agents. This has occasioned a great effort in the search for novel bioactive agents, including available natural products and their derivatives.

Naturally occurring diterpenoids with a dehydroabietane skeleton (ketones, alcohols and phenols) are often discovered and isolated from plants and have been reported for their bioactivity [1], [2], [3]. While the isolation of natural products from plants or animals is a useful way for the discovery of profitable drugs, hemisynthesis or derivatisation of natural products can be a faster and economical approach to the search for biologically active compounds [4], [5], [6]. Following previous studies on the synthesis [5] and bioactivity [6] of C-7 oxidised dealkylated resin acid derivatives against fungi and Gram-positive bacteria, we report herein attempts to establish the structural features that could influence the biological activity of natural identical and synthetic diterpenes with a hydrophenanthrene skeleton.

In this context, simple and sequential modifications were performed in the molecule of dehydroabietic acid **1,** compound **1** and 14 derivatives with or without an isopropyl group at C-13, different stereoconfiguration in the A/B ring junction (*cis* or *trans*) and different functional groups, such as acid, ester, alcohol or aldehyde at C-18, and also at C-7 or C-12 were tested. All the compounds were easily obtained in good yield, by standard chemical procedures. In preliminary bioassays, the microorganisms tested were a filamentous fungus, yeasts, and bacteria.

Some of the synthesized compounds are new and were fully characterised.

Affiliation
[1] Departamento de Tecnologia de Indústrias Quimicas and Laboratório de Microbiologia Industrial, Instituto Nacional de Engenharia e Tecnologia Industrial, Lisboa, Portugal
[2] Departamento de Química, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal

Correspondence
Bárbara Gigante, Ph. D. · INETI, Departamento de Tecnologia de Indústrias Químicas · Estrada do Paço do Lumiar, 22, 1649-038 Lisbon · Portugal · E-Mail: barbara.gigante@ineti.pt · Fax: +351.217.168.100

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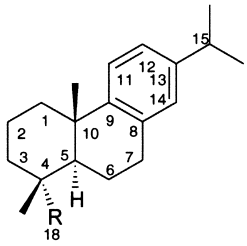
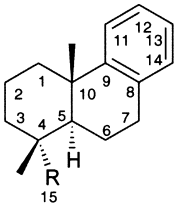
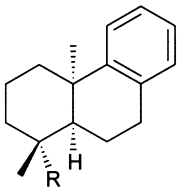


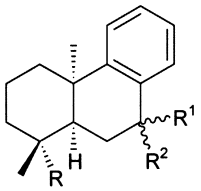
1 - R=CO_2H
2 - R=CO_2Me
3 - R=CH_2OH
4 - R=CHO



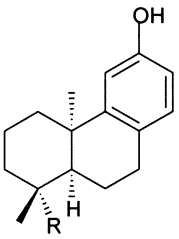
5 - R=CO_2H
6 - R=CO_2Me
7 - R=CH_2OH
8 - R=CHO



9 - R=CO_2H
10 - R=CO_2Me
11 - R=CH_2OH
12 - R=CHO



13 - R=CO_2Me, R^1,R^2 = O
14 - R=CH_2OH, R^1=OH, R^2 = H
15 - R=CHO, R^1,R^2= O



16 - R=CO_2Me
17 - R=CH_2OH

Materials and Methods

Chemistry

Melting points were determined on a Reichert Thermovar melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 457 spectrophotometer. NMR spectra were recorded on a General Electric QE-300 spectrometer at 300.65 MHz for ^{1}H and at 65.7 MHz for ^{13}C, using a 5-mm dual probe with $CDCl_3$ as solvent. The chemical shifts reported are in δ (ppm, TMS). EIMS were recorded on a Kratos MS 25RF mass spectrometer at 70 eV and HREIMS were determined on Extrell (Waters) FTMS 2001-DT S.T.I.C.R. instrument. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Elemental analyses were performed on a Carlo Erba 1106 elemental analyser. TLC was carried out on Merck precoated silica gel sheets (GF_{254}). Column chromatography was performed using Merck 60 silica gel (230–400 mesh). UV light was used for the detection of compounds on TLC. All solvents were purified by standard methods and anhydrous solvents were dried and freshly distilled.

Dehydroabietic acid (abieta-8,11,13-trien-18-oic acid) (**1**): Obtained from dehydrogenated commercial rosin [7]. Recrystallization from methanol/water gave colourless prisms, m.p. 166–168 °C (lit. [7]: 162–165 °C; lit. [8]: 167–169 °C); $[\alpha]_D^{25}$: +58° (*c* 0.50, $CHCl_3$) {lit. [8]: $[\alpha]_D^{25}$ +57.3° (*c* 1.04, $CHCl_3$)}.

Methyl dehydroabietate (methyl abieta-8,11,13-trien-18-oate) (**2**): Obtained by methylation of **1** with diazomethane. The crude product affords by recrystallization from methanol colourless prisms, m.p. 63–65 °C (lit. [8]: 64–65 °C); $[\alpha]_D^{25}$: +53° (*c* 0.51, $CHCl_3$) {lit. [8]: $[\alpha]_D^{25}$: +50.6° (*c* 0.9, $CHCl_3$) EtOH)}.

10β-Methyl-13-deisopropyldehydroabietic acid (10β-methyl-8,11,13-podocarpatrien-15-oic acid) (**5**) *and 10α-methyl-13-deisopropyldehydroabietic acid (10α-methyl-8,11,13-podocarpatrien-15-oic acid* (**9**): Prepared, as a mixture, by dealkylation of **1** with $AlCl_3$ [9]. Repeated recrystallizations from MeOH, allowed the separation of both isomers as colourless crystals, **5** (25%), m.p. 161–162 °C (lit. [9]: 161–163 °C); $[\alpha]_D^{25}$: +63° (c 0.51, $CHCl_3$) {lit. [9]: $[\alpha]_D^{25}$: +69.4° c 2.7} and **9** (60%), m.p. 172–173 °C (lit. [9]: 171–173 °C); $[\alpha]_D^{25}$: –1.2° (c 0.51, $CHCl_3$) {lit. [9]: $[\alpha]_D^{25}$: +1.7° (c 2.96)}.

Methyl 10β-methyl-13-deisopropyldehydroabietate (**6**): Methylation of **5** with CH_2N_2 or dealkylation of **2** with HY zeolite [10] afforded **6** (quantitative yield or 75%, respectively) as colourless crystals from methanol, m.p. 107–109 °C (lit. [9]: 108–109 °C); $[\alpha]_D^{25}$: +58.3° (*c* 0.55, $CHCl_3$) {lit. [9]: $[\alpha]_D^{25}$: +60.5° (*c* 2.2)}.

Methyl 10α-methyl-13-deisopropyldehydroabietate (**10**): Methylation of **9** with CH_2N_2, afforded **10** (quantitative yield) as colourless crystals from methanol, m.p. 92–94 °C (lit. [9]: 93–94 °C); $[\alpha]_D^{25}$: –4.3° (*c* 0.31, $CHCl_3$) {lit. [9]: $[\alpha]_D^{25}$: –5.1° (*c* 2.94)}.

Methyl 10α-methyl-7-oxo-13-deisopropyldehydroabietate (**13**): Photoxidation of **10** [5] gave **13** (90%) as a colourless gum recrystallized from MeOH, m.p. 75–77 °C (lit. [5]: colourless oil); $[\alpha]_D^{25}$: –79° (*c* 0.2, $CHCl_3$) {lit. [9]: $[\alpha]_D^{25}$: –78.9° (*c* 2.2)}.

Methyl 12-hydroxy-10α-methyl-13-deisopropyldehydroabietate (**16**): Obtained from **10** following a procedure early described [11] as colourless needles from diethyl ether, m.p. 123–125 °C (lit. [11]: 121–122 °C); Anal. calcd. for $C_{18}H_{24}O_3$ requires C 75.20%, H 8.48%; found: C 75.22%, H 8.45%; $[\alpha]_D^{25}$: –12° (*c* 0.51, $CHCl_3$).

Reductions of **2**, **10**, **13** and **16**: Carried out with $LiAlH_4$ in anhydrous ether under N_2 atmosphere, by a previously described procedure [8].

Dehydroabietinol (**3**): (98% from **2**), viscous oil (lit. [8], [11]: oil); IR (film): ν_{max} = 3320 (OH) cm^{-1}; $[\alpha]_D^{25}$: +52° (*c* 0.55, $CHCl_3$) {lit. [8]: $[\alpha]_D^{25}$: +51.9° (*c* 0.45, $CHCl_3$)}.

10β-Methyl-13-deisopropyldehydroabietinol (**7**): (95% from **6**), colourless oil (lit. [12]: 85–96 °C]; $[\alpha]_D^{25}$: +53° (*c* 0.54, $CHCl_3$); IR (film) ν_{max} = 3320 (OH) cm^{-1}; EIMS: *m/z* (rel. int.) = 244 $[M]^+$ (17), 229 $[M-CH_3]^+$ (55), 211 $[M-CH_3-H_2O]^+$ (75), 131 (100).

10a-Methyl-13-deisopropyldehydroabietinol (**11**): (98% from **10**), colourless needles from petroleum ether, m.p. 68–69 °C; $[\alpha]_D^{25}$: –12.2° (*c* 0.35, $CHCl_3$); IR (KBr): ν_{max} = 3320 (OH) cm^{-1}; EIMS: *m/z* (rel. int.) = 244 $[M]^+$ (17), 229 $[M-CH_3]^+$ (55), 211 $[M-CH_3-H_2O]^+$ (75), 131 (100); ^{1}H-NMR ($CDCl_3$) δ = 0.32 (3H, s, 4-CH_3), 1.19 (3H, s, 10-CH_3), 1.19–1.24 (1H, m, 3-Hα), 1.32–1.42 (1H, m, 1-Hα), 1.47–1.57 (3H, m, 2-H_2, 3-Hβ), 1.79 (1H, dd, *J* = 7.4, 2.0 Hz, 5-H), 1.88–1.98 (1H, m, 6-Hβ), 2.15–2.28 (1H, m, 6-Hα), 2.42–2.48 (1H, m, 1-Hβ), 2.80–3.00 (2H, m, 7-H_2), 3.20 (1H, AB system, *J* = 11.0 Hz, 4-C$\underline{H}_2$OH), 3.53 (1H, AB system, *J* = 11.0 Hz, 4-C$\underline{H}_2$OH), 4.76 (1H, brs, D_2O exchange, 4-$CH_2O\underline{H}$), 7.01–7.16 (3H, m, Ar-H), 7.27–7.30 (1H, m, Ar-H); ^{13}C-NMR ($CDCl_3$): δ = 37.5 (C-1), 17.4 (C-2), 36.6 (C-3), 38.8 (C-4), 43.1 (C-5), 18.5 (C-6), 26.0 (C-7), 137.2 (C-8), 143.7 (C-9), 36.9 (C-10), 123.6 (C-11), 125.1 (C-12), 125.4 (C-13), 128.7 (C-14), 72.0 (C-4-$\underline{C}H_2$OH), 18.2 (C-4-$\underline{C}H_3$), 34.1 (C-10-$\underline{C}H_3$); Anal. calcd. for $C_{17}H_{24}O$ requires C 83.55, H 9.90%; found: C 83.52, H 9.92%.

7-Hydroxy-10α-methyl-13-deisopropyldehydroabietinol (**14**): (95% from **13**) colourless oil; $[\alpha]_D^{25}$: −55° (*c* 0.26, $CHCl_3$); HREIMS: *m/z* = 260.1745 $[M]^+$ (calcd. for $C_{17}H_{24}O_2$: 260.1770); IR (neat): ν_{max} = 3400 (OH) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ = 0.28 (3H, s, 4-CH_3), 1.21 (3H, s, 10-CH_3), 1.21 – 1.28 (1H, m, 3-Hα), 1.45 – 1.62 (4H, m, 1-Hα, 2-H_2, 3-Hβ), 1.66 – 1.75 (1H, m, 6-Hβ), 1.70 – 1.90 (1H, brd, *J* = 11.0 Hz, D_2O exchange, 4-CH_2O<u>H</u>), 1.88 – 1.93 (1H, dd, *J* = 7.8, 4.5 Hz, 5-H), 2.26 – 2.31 (1H, m, 1-Hβ), 2.40 (1H, brs, D_2O exchange, 7-O<u>H</u>), 2.62 (1H, td, 6-Hα), 3.30 (1H, AB system, *J* = 11.0 Hz, 4-C<u>H_2</u>OH), 3.47 (1H, AB system, *J* = 11.0 Hz, 4-C<u>H_2</u>OH), 4.91 – 4.98 (1H, m, t after D_2O exchange, 7-H), 7.20 – 7.30 (3H, m, Ar-<u>H</u>), 7.56 – 7.60 (1H, t, Ar-H); ^{13}C-NMR ($CDCl_3$) δ = 37.0 (C-1), 18.3 (C-2), 34.9 (C-3), 38.7 (C-4), 41.8 (C-5), 31.4 (C-6), 66.1 (C-7), 139.8 (C-8), 143.7 (C-9), 37.5 (C-10), 124.1 (C-11), 124.6 (C-12), 125.6 (C-13), 126.9 (C-14), 71.5 (C-4-<u>CH_2</u>OH), 19.8 (C-4-<u>CH_3</u>), 32.0 (C-10-<u>CH_3</u>); EIMS: *m/z* (rel. int.) = 260 $[M]^+$ (26), 242 $[M-H_2O]^+$ (5), 224 $[M-2H_2O]^+$ (8), 155 (100), 141 (74), 128 (45), 115 (51).

12-Hydroxy-10α-methyl-13-deisopropyldehydroabietinol (**17**): (95% from **16**) as colourless needles from Et_2O/petroleum ether, m.p. 155 – 157 °C; $[\alpha]_D^{25}$: −13° (*c* 0.22, $CHCl_3$); IR (KBr): ν_{max} = 3470 (OH), 3190 (OH) cm^{-1}; EIMS: *m/z* (rel. int.) = 260 $[M]^+$ (59), 245 $[M-CH_3]^+$ (36), 227 $[M-CH_3-H_2O]^+$ (100), 211 (24), 171 (31), 165 (40), 137 (40), 115 (73), 89 (100); ^{1}H-NMR ($CDCl_3$) δ = 0.36 (3H, s, 4-CH_3), 1.17 (3H, s, 10-CH_3), 1.18 – 1.24 (1H, m, 3-Hα), 1.32 – 1.40 (1H, m, 1-Hα), 1.48 – 1.56 (3H, m, 2-H_2, 3-Hβ), 1.76 (1H, dd, *J* = 7.2, 1.8 Hz, 5-H), 1.86 – 1.95 (1H, m, 6-Hβ), 2.11 – 2.24 (1H, m, 6-Hα), 2.35 (1H, d, *J* = 12.9 Hz, 1-Hβ), 2.58 (1H, brs, D_2O exchange, 4-CH_2O<u>H</u>), 2.78 – 2.84 (2H, m, 7-H_2), 3.21 (1H, AB system, *J* = 11.1 Hz, , after D_2O exchange, 4-C<u>H_2</u>OH), 3.53 (1H, AB system, *J* = 11.1 Hz, after D_2O exchange, 4-C<u>H_2</u>OH), 6.58 (1H, dd, *J* = 8.1, 2.4 Hz, 13-H), 6.79 (1H, dd, *J* = 2.4 Hz, 11-H), 6.88 (1H, dd, *J* = 8.1 Hz, 14-H), 8.01 (1H, s, D_2O exchange, 12-OH); ^{13}C-NMR ($CDCl_3$) δ = 37.6 (C-1), 17.5 (C-2), 36.7 (C-3), 38.9 (C-4), 42.9 (C-5), 18.6 (C-6), 25.1 (C-7), 128.0 (C-8), 145.0 (C-9), 36.9 (C-10), 110.7 (C-11), 154.6 (C-12), 112.3 (C-13), 129.3 (C-14), 71.5 (C-4-<u>CH_2</u>OH), 18.2 (C-4-<u>CH_3</u>), 34.2 (C-10-<u>CH_3</u>); Anal. calcd. for $C_{17}H_{24}O_2$ requires C 78.42, H 9.29%; found: C 78.44, H 9.28%.

Oxidation of **3**, **11** and **14**: Done with PCC in dichloromethane in accordance with a procedure previously described [8]. Pure aldehydes were obtained after column chromatography (silica gel, petroleum ether with gradual addition of dichloromethane) and recrystallization from methanol/petroleum ether:

Desidroabietinal (**4**): (94% from **3**), colourless needles from methanol/petroleum ether, m.p. 85 – 87 °C (lit. [8]: 86 – 88 °C); $[\alpha]_D^{25}$: +56° (*c* 0.51, $CHCl_3$) {lit. [12]: $[\alpha]_D^{25}$ +55.8° (*c* 0.95, $CHCl_3$)}.

10α-Methyl-13-deisopropyldehydroabietinal (**12**): (94% from **11**), thick gum from petroleum ether (lit. [14]: oil, bp 85 °C/0.7 Torr); $[\alpha]_D^{25}$: −1.2° (*c* 0.24, $CHCl_3$) {lit. [14]: $[\alpha]_D^{25}$: −1.8° (*c* 1.6, EtOH)}; HREIMS: *m/z* = 242.1659 $[M]^+$ (calcd. for $C_{17}H_{22}O$: 242.1670); IR (KBr): ν_{max} = 1715 (C = O) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ = 0.88 (3H, s, 4-CH_3), 1.23 (3H, s, 10-CH_3), 1.15 – 1.25 (1H, m, H-3α), 1.47 – 1.70 (4H, m, 6-Hβ, 2-Hβ, 1-Hα, 5-H), 1.77 – 1.92 (2H, m, 1-Hβ, 2-Hα), 1.97 – 2.08 (2H, m, 3-Hβ, 6-Hα), 2.85 – 2.89 (2H, m, 7-H_2), 7.03 – 7.17 (3H, m, Ar-H), 7.25 – 7.28 (1H, m, Ar-H), 9.55 (1H, d, *J* = 1.2 Hz, 4-C<u>H</u>O); ^{13}C-NMR ($CDCl_3$): δ = 36.4 (C-1), 18.9 (C-2), 30.0 (C-3), 49.5 (C-4), 45.2 (C-5), 19.9 (C-6), 29.3 (C-7), 135.9 (C-8), 145.6 (C-9), 38.2 (C-10), 125.4 (C-11), 125.9 (C-12), 126.1 (C-13), 128.7 (C-14), 206.5 (C-4-<u>C</u>HO), 21.3 (C-4-<u>CH_3</u>), 28.3 (C-10-<u>CH_3</u>); EIMS: *m/z* (rel. int.) = 242 $[M]^+$ (5), 227 $[M-CH_3]^+$ (17), 209 (23), 143 (42), 131 (100).

10α-Methyl-7-oxo-13-deisopropyldehydroabietinal (**15**): (91% from **14**), colourless needles from CH_2Cl_2/*n*-hexane, m.p. 219 – 221 °C; $[\alpha]_D^{25}$: −60° (*c* 0.26, $CHCl_3$); HREIMS: *m/z* = 256.1452 $[M]^+$ (calcd. for $C_{17}H_{20}O_2$: 256.1463); IR (KBr): ν_{max} = 1775 (C = O), 1725 (C = O) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ = 0.59 (3H, s, 4-CH_3), 1.36 (3H, s, 10-CH_3), 1.20 – 1.42 (1H, m, 3-Hα), 1.49 – 1.69 (4H, m, 3-Hβ, 2-H_2, 1-Hα), 2.38 – 2.53 (3H, m, 6-Hα, 5-H, 1-Hβ), 3.07 (1H, dd, *J* = 18.9, 6.6 Hz, 6-Hβ), 7.31 – 7.40 (2H, m, Ar-H), 7.54 – 7.60 (1H, m, Ar-H), 8.04 (1H, dd, *J* = 7.8, 1.2 Hz, Ar-H), 9.34 (1H, s, 4-C<u>H</u>O); ^{13}C-NMR ($CDCl_3$) δ = 36.4 (C-1), 17.5 (C-2), 32.7 (C-3), 50.3 (C-4), 43.6 (C-5), 37.1 (C-6), 197.2 (C-7), 132.7 (C-8), 148.9 (C-9), 36.6 (C-10), 124.3 (C-11), 126.4 (C-12), 127.4 (C-13), 134.2 (C-14), 205.1 (C-4-<u>C</u>HO), 16.4 (C-4-<u>CH_3</u>), 34.2 (C-10-<u>CH_3</u>); FAB-MS: *m/z* (rel. int.) = 257 $[M+H]^+$ (46), 239 (19), 227 (21), 211 (24), 171 (31), 165 (40), 137 (40), 115 (73), 89 (100).

Biological evaluation

In vitro antimicrobial activity: The microorganisms assayed were *Trichophyton mentagrophytes* CCMI 226, *Staphylococcus aureus* CCM I335, *Serratia marcescens* CCMI 638, *Pseudomonas aeruginosa* CCMI 331, *Candida parapsilosis* CL 2, *Candida kruzei* CL 6 and two strains of *Candida albicans*, CMI 209 and CMI 110. The bioassays with a filamentous fungus were performed according to Henricks [14], using compounds dissolved in acetone and tested at 0.1% (w/w, compound/culture medium). Blank plates containing acetone were equally prepared. Amphotericin B was used as positive control. The results were expressed as Relative Inhibition (RI).

Yeast and bacteria bioassays were performed on multiwell plates according to a previously described procedure [6]. The test compounds, dissolved in acetone at a concentration of 50 mg/ml, were applied to the multiwell plates containing the microorganism incorporated in the culture medium. 5-Fluorocytosine and rifampicin were used as positive controls, respectively, for yeasts and bacteria.

Results and Discussion

Synthesis of the compounds

The dehydroabietic acid **1**, isolated from dehydrogenated rosin (65 – 70% yield [7]), was the starting material from which all the others compounds, natural identical (**2 – 4**) [15], [16], [17], [18], [19] or synthetic (**5 – 7** and **9 – 17**) diterpenes were successively obtained.

The dealkylated isomers, 10β-methyl-13-deisopropyldehydroabietic acid **5** (25%) and 10α-methyl-13-deisopropyldehydroabietic acid (**9**) (60%) were obtained from **1** by a Friedel-Crafts reaction with $AlCl_3$ in toluene [11]. Methylation of the acids **1, 5** and **9** with diazomethane gave the corresponding esters **2, 6** and **10** in quantitative yield. Methyl 10β-methyl-13-deisopropyldehydroabietate (**6**) can also be obtained selectively (75%) by dealkylation of **2** using an acidic HY zeolite as catalyst [10].

In order to improve the activity of these compounds additional functions were introduced. Methyl 10α-methyl-7-oxo-13-deiso-

propyldehydroabietate (**13**) and the phenol, methyl 12-hydroxy-10α-methyl-13-deisopropyldehydroabietate (**16**) were obtained from **10**, respectively, by a selective photo-oxygenation procedure [5] or by a sequential acetylation, Bayer-Villiger oxidation and hydrolysis methodology [11]. Due to the differences in the experimental procedure used and in the melting point of the compound obtained with that previously reported in the literature, the characterisation of **16** was also done. Esters **2, 6, 10, 13** and **16** on reduction with lithium aluminium hydride ($LiAlH_4$) furnished the respective alcohols, **3, 7, 11, 14** and **17**, which by oxidation with pyridinium chlorochromate (PCC) gave the respective aldehydes **4, 8, 12** and **15**. 10β-Methyl-13-deisopropyldehydroabietinal (**8**) could not be characterised and tested due to its quick degradation during the purification step.

The new compounds (**11, 14, 15** and **17**) and the aldehyde **12** were characterised by different physical and spectrometric methods (m.p., IR, NMR, MS and/or elemental analysis), while the structures of the other compounds, already known and described, were established by comparison with data found in the literature.

Antimicrobial activity assays

The antimicrobial activity of deisopropylated resin acid derivatives **5 – 7** and **9 – 12** was tested and compared with that of C-13 isopropylated analogous **1 – 4**. The results were also compared with those obtained with the bifunctional derivatives **13 – 15** and **17** (Table **1**).

With the filamentous fungus the bioassays were performed in acetone solution using the solvent as control [1] and the results are expressed as relative inhibition RI (%). For yeasts and bacteria a multiwell plates assay was performed according to a procedure previously described [6] and the results expressed in terms of minimum inhibitory concentration MIC (μmol/ml). The amounts of compounds tested by these techniques are high and are related with the density of the microbial population used in the test, 10^8 CFU/ml for fungi and 10^5 CFU/ml for bacteria [1], [6].

As summarized in Table **1**, all compounds showed activity against *T. mentagrophytes*. Antifungal activity is enhanced by the alcohols without an isopropyl group at C-13, **7** and **11** showing RI of 91% and 90%, which improved to 100% when another hydroxy group is present in the molecule (**14** and **17**). No significant differences in activity against *T. mentagrophytes* were induced by the difference on the A/B ring junction (**5 – 7** vs. **9 – 11**). In acetone the microorganisms grew normally, while amphotericin B inhibited the fungal growth (RI = 100%).

The assays against several *Candida* strains show that only the compounds containing an aldehyde group at C-18 (**4**, **12** and **15**) inhibited their growth. The dehydroabietinal **4** shows the same MIC (> 70 μmol/ml) for all the strains, decreasing significantly for the deisopropylated analogue with a *cis* A/B ring junction, **12**, with MIC between 26.9 and 53.8 μmol/ml. That value is twofold reduced for *C. albicans* 110, *C. albicans* 407, *C. kruzei*, or even lower (6.3 μmol/ml) for *C. parapsilosis* when the aldehydic compound possesses a ketone group at C-7 (**15**). In spite of this increasing effect on the activity, those compounds are less active than the control (5-fluorocytosine) which showed a lower MIC (0.8 μmol/ml).

Table **1** Bioactivity of dehydroabietic acid derivatives against a filamentous fungus (F.F.), yeasts and bacteria[a,b]

	F.F.[c] *RI (%)*	Yeasts[d,e] *(µmol/ml)*				Bacteria[d),f)] *(µmol/ml)*
Compound	T. mentagrophytes	C. albicans *110*	C. albicans *407*	C. kruzei	C. parapsilosis	S. aureus
1	6.8	na	na	na	na	na
2	7.0	na	na	na	na	na
3	56	na	na	na	na	5.6
4	44	> 70	> 70	> 70	> 70	5.6
5	77	na	na	na	na	na
6	78	na	na	na	na	na
7	91	na	na	na	na	6.5
9	77	na	na	na	na	na
10	49	na	na	na	na	na
11	90	na	na	na	na	6.5
12	nt	26.9	26.9	53.8	26.9	6.6
13	88	na	na	na	na	22.7
14	100	na	na	na	na	3.5
15	39	12.7	12.7	25.4	6.3	3.9
17	100	na	na	na	na	1.5
Amphotericin B	100	nt	nt	nt	nt	nt
5-Fluorocytosine	nt	0.8	0.8	0.8	0.8	nt
Rifampicin	nt	nt	nt	nt	nt	0.001

[a] No inhibition of growth was observed at 40 μmol/ml for any of the tested compounds against the Gram-negative bacteria, *P. aeroginosa* and *S. marcencens*, also tested.
[b] nt – not tested.
[c] Acetone as control.
[d] Values of MIC (μmol/ml.
[e] na – not active below 80 μmol/ml.
[f] na – not active below 40 μmol/ml.

In the case of bacteria, none of the tested compounds inhibited the growth of Gram-negative bacteria *P. aeruginosa* and *S. marcescens*. The presence of alcohols or aldehydes at C-4 (**3**, **4**, **7**, **11** and **12**) seems to be important for the inhibition of Gram-positive bacteria *S. aureus*. MIC has quite similar values (*ca.* 6 μmol/ml) for compounds either with (**3**, **4**) or without the isopropyl group at C-13 (**7**, **11**, **12**), and is independent of the stereoconfiguration (**3**, **4**, **7** vs. **11**, **12**). The MIC is reduced two-fold when another hydroxy or ketone group is present in the molecule (**14**, **15**) at C-7 or C-12. For *S. aureus* the activity of rifampicin, used as a control, showed a lower MIC (0.001 μmol/ml) than the compounds under study.

The lack of activity of dehydroabietic acid derivatives towards Gram-negative bacteria has already been observed [1], [6]. In a previous work [6], only combined methyl 10α-methyl-7-oxo-13-deisopropyldehydroabietate (**13**) and its 10β-methyl isomer inhibited the growth of *Escherichia coli* and *Klebsiella pneumoniae*.

Comparison and correlation with other studies [1], [2], [3], where the bioactivity of diterpenes with a dehydroabietane skeleton was evaluated, is not straightforward, mainly due to the difference on the biological methodologies used.

From this study we can conclude that simple derivatisation of resin acid derivatives can increase their bioactivity. The alcohol or aldehyde C-18 derivatives of dehydroabietic acid are more active against *T. mentagrophytes*, a filamentous fungus, several *Candida* strains (*C. albicans* 110, *C. albicans* 407, *C. kruzei*, *C. parapsilosis*) and *S. aureus*, than the acid or its methyl ester themselves. Their activity can be increased by deisopropylation or by the introduction of an alcohol or ketone function at C-7 or C-12, depending on the micro-organism under study. The stereochemistry of the A/B ring junction seems not to display a significant role.

Nevertheless, the low activity found for the compounds under study, the structural effects observed in this work may be an important basis for further investigations on resin acid derivatives towards bioactive compounds.

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