Short Communication

Novel Bioactive Tropinone Derivatives from Sesquiterpenoid Unsaturated Dialdehydes via the Robinson–Schöpf Reaction

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Natural products continue to play an important role as a source of novel bioactive compounds and crude extracts as well as pure natural products are included in most screening programs for new bioactive compounds. A number of natural compounds are not useful, because the bioactivity is caused by their reactivity as electrophiles. Such compounds are often toxic and although they may be very potent in one bioassay they are generally not selective. While the reactivity is normally caused by a chemical functionality, the remaining part of the molecule may play an important role in modulating the reactivity and/or directing the compound to selected targets. Examples are the terpenoid unsaturated dialdehydes isolated from a number of natural sources, e.g., the marasmic (+)-marasmic acid (1a) and the isolactaranic (−)-merulidial (2) isolated from the fungi Marasmius conigenus and Merulius tremellosus, respectively (Fig. 1). Marasmic acid (1a), originally isolated because of its antibacterial activity, a and methyl marasmic (1b) strongly interfere with eukaryotic nucleic acid biosyntheses, e.g., RNA polymerase II and capping enzyme (mRNA guanylyltransferase), b while merulidial (2), a weak antibacterial and antifungal agent, c is a potent inducer of differentiation of human promyelocytic leukemia (HL-60) cells. d To a large degree, the bioactivities of these compounds depend on the presence of a reactive α,β-unsaturated 1,4-dialdehyde functionality, but it has been shown that small structural changes may influence the bioactivities of the unsaturated dialdehydes strongly e and that the activities of structural isomers and even of enantiomers may be very different. f We therefore sought suitable ways to transform reactive and enantiomerically pure fungal metabolites with a certain degree of structural complexity and available in reasonable amounts by fermentation [e.g., methyl marasmate (1b) and merulidial (2)], into novel compounds. The transformations should change the reactive functionality (i.e., the unsaturated dialdehyde) in good yields into something that lacks electrophilic but is associated with bioactivity. In this study, we have used the classical

Fig. 1. R = H; b: R = CH₃.
Robinson–Schöpf reaction\(^9\) and prepared novel tropinone derivatives from methyl marasmate (1b) and merulidial (2). Preliminary biological characterisation of the products has indicated that they are inhibitors of a cellular signal transduction pathway.

Results and discussion

Virtually all Robinson–Schöpf reactions previously reported have been carried out with butanediol in aqueous solution,\(^9\) but as the terpenoid dialdehydes to be used have very limited water solubility it was necessary to develop other reaction conditions. Attempts were made in a number of solvents, and for this investigation THF was found to be the most suitable.

The two tropinones 3 and 4 were obtained in 74% overall yield from methyl marasmate (1b) (prepared by treatment of 1a with diazomethane as described previously\(^6\)), and the diastereomers could be separated by chromatography. The ratio between the two was approximately 3:2 (determined from the integrals in the \(^1\)H NMR spectrum of the crude product), and temperature independent. The reaction did not work well with marasmic acid (1a) itself, probably due to the fact that it is present in solution as the hemiacetal, and complex mixtures of products were obtained. Merulidial (2) gave the tropinones 5 and 6 (ratio 7:1) in 51% overall yield. Due to the very similar chromatographic properties of the two isomers 5 and 6, the minor isomer was not obtained completely pure by HPLC. In the NMR spectra of the mixture of 5 and 6 all the expected signals for 6 were present. The structures of the tropinones were determined by 2D NMR experiments, and the assignments made in the Experimental are based on correlations observed in COSY, HMOC and HMBC spectra. The stereostructure was determined from NOESY data, e.g., correlations between 1-H2 and 19-H2 in 3, between 19-H3 and 13-H3 as well as between 6-Hz and 15-Hz in 4, and between 1’-H2 and 17’-H2 in 5.

Preliminary assays of the cytotoxic activity of the tropinone derivatives indicate that the derivatisation of the reactive unsaturated dialdehyde moiety as expected reduces the cytotoxicity of the parent compounds.\(^10\) Interestingly, the tropinones reported here have inhibitory activity in a signal transduction pathway currently studied in one of our laboratories,\(^11\) indicating that they may possess, for example, anti-inflammatory activity. Studies of this and other biological activities of the tropinone derivatives are under way, and will be reported elsewhere.

In conclusion, novel pentacyclic tropinone derivatives with specific biological activity on mammalian cells can be prepared from sesquiterpenoid unsaturated dialdehydes, by the classical three-component Robinson–Schöpf reaction.

Experimental

TLC analyses were carried out on Merck DC-Alufolie Kieselgel 60 F254 SiO2 plates, visualised by spraying with anisaldehyde–sulfuric acid and heating to 120 °C. The EIMS spectrum (direct inlet, 70 eV) was recorded with a JEOL SX102 spectrometer, and the NMR spectra (for samples in CDCl\(_3\)) with a Bruker ARX 500 spectrometer. The chemical shifts are reported in ppm, with the solvent signals (\(\delta_\text{HM} = 7.26\) and \(\delta_\text{C} = 77.0\)) as reference, and the coupling constants (\(J\)) are given in Hz. COSY, HMOC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For the 2D heteronuclear correlation spectroscopy the refoocusing delays were optimised for \(1\;T_{\text{CH}} = 145\;\text{Hz}\) and \(J_{\text{CH}} = 10\;\text{Hz}\). The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (rev. 941001). The optical rotations were measured with a Perkin–Elmer 141 polarimeter at ambient temperature. Marasmic acid (1a)\(^12\) and merulidial (2)\(^3\) were isolated as described previously.

General procedure for the Robinson–Schöpf reaction. To a stirred solution of the dialdehyde (0.1 mmol) in THF (1 ml) at ambient temperature, acetonedicarboxylic acid (0.11 mmol) was added followed by propylamine (0.11 mmol). The reaction mixture was stirred for 72 h, whereafter the solvent was evaporated off and the residue was subjected to chromatography on SiO\(_2\) with heptane–EtOAc as the eluent. The diastereomeric mixture was obtained as a yellow oil. The diastereomers were separated by HPLC with a silica column (500 x 10 mm, 10 µm) using hexane–tert-butyl methyl ether–2-propanol (4.5 ml min\(^{-1}\)) mixtures as the eluent.

\((1R, 2R, 4S, 5S, 9S, 12S)-4-Methoxy carbonyl-7,7-dimethyl-16-propyl-16-azapentacyclo [10.3.1.0\(^6\).0\(^{10}\).0\(^{12}\).0\(^{14}\).0\(^{15}\)] hexadec-10-ene-14-one (3) was obtained as a colourless oil. \([\alpha]\)\(_D\) = 109\(^\circ\) (c 1.00, CHCl\(_3\)). Ei-MS, m/z (% rel. int.): 357.2289 (M\(^+\)), 228 (100, C\(_2\)H\(_4\)). NO\(_3\) requires 357.2304, 328 (31), 314 (97), 300 (31), 298 (58), 258 (17), 176 (20), 28 (86). \(6\) H NMR (500 MHz, CDCl\(_3\)): 6 5.14 (1H, s, H-10), 3.75 (1H, m, H-12), 3.71 (1H, m, H-1), 3.70 (3H, s, H-20), 2.77 (1H, m, H-5), 2.75 (1H, d, \(J_{13-15} = 4.4\)), 2.59 (1H, d, \(J_{13-15} = 16.3\), H-13\(\beta\)), 2.72 (1H, d, \(J_{19-18} = 3.9\), \(J_{15-16} = 16.5\)), 2.48 (1H, m, H-15\(\beta\)), 2.46 (1H, m, H-9), 2.45 (2H, m, H-1\(\beta\)), 2.37 (1H, d, \(J_{13-12} = 16.3\), H-13\(\alpha\)), 1.93 (1H, dd, \(J_{6b-5} = 7.2\), \(J_{6b-6a} = 12.7\), H-6\(\beta\)), 1.75 (1H, dd, \(J_{6a-6b} = 7.6\), \(J_{6b-6a} = 13.2\), H-6\(\beta\)), 1.48 (2H, sext, \(J_{2-3} = 7.4\), H-2\(\alpha\)), 1.41 (1H, d, \(J_{6a-6b} = 13.2\), H-8\(\beta\)), 1.36 (1H, dd, \(J_{6b-5} = 12.4\), \(J_{6a-6b} = 12.6\), H-6\(\alpha\)), 1.31 (1H, d, \(J_{3b-3a} = 4.3\), H-3\(\beta\)), 1.09 (1H, d, \(J_{3a-3b} = 4.4\), H-3\(\alpha\)), 1.03 (3H, s, H-18), 0.98 (3H, s, H-19), 0.90 (3H, t, \(J_{2-3} = 7.4\), H-3\(\alpha\)). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): 6 209.5 (C-14), 174.2 (C-17), 143.2 (C-11), 120.6 (C-10), 64.2 (C-12), 60.4 (C-1), 55.3 (C-51), 51.7 (C-20), 48.5 (C-15), 48.4 (C-13), 47.3 (C-8), 44.2 (C-6), 39.1 (C-9), 38.3 (C-5), 37.5 (C-7), 35.3 (C-2), 34.6 (C-4), 32.3 (C-18), 31.9 (C-19), 23.2 (C-3), 21.8 (C-2), 11.9 (C-3).
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(1S, 2R, 4S, 5S, 9S, 12R)-4-Methoxycarbonyl-7,7-dimethyl-16-propyl-16-azapentacyclo [10.3.1.0^2,0^6,12]hexadec-10-ene-14-one (4) was obtained as a yellow oil, [a]_D^20 = -157° (c 0.41, CHCl_3). EI-MS, m/z (% rel. int.): 357.2299 (M^+), 100, C_23H_19NO_3 requires 357.2304, 328 (31), 314 (97), 300 (31), 298 (58), 218 (17), 176 (20), 28 (86). ^1H NMR (500 MHz, CDCl_3): δ 5.12 (1H, s, H-10), 3.88 (1H, m, H-12), 3.67 (3H, s, H-20), 3.63 (1H, m, H-1), 2.79 (1H, ddd, J_{5,6b}=7, J_{5,6a}=7, J_{5,6a}=12.0, H-5), 2.75 (1H, m, H-13β), 2.64 (2H, m, H-1’), 2.59 (1H, m, H-15β), 2.52 (1H, m, H-9), 2.34 (1H, d, J_{13b-13a}=16.3, H-13α), 2.19 (1H, d, J_{15u-15b}=17.3, H-15α), 1.88 (1H, dd, J_{6b-6a}=7.3, J_{6b-6a}=12.9, H-6β), 1.72 (1H, dd, J_{8b-8a}=7.2, J_{8b-8a}=13.2, H-8β), 1.59 (2H, m, H-2’), 1.51 (1H, d, J_{3b-3a}=3.5, H-3β), 1.37 (1H, m, H-8α), 1.34 (1H, d, J_{3a-3b}=3.5, H-3α), 1.02 (3H, s, H-18), 1.00 (1H, m, H-6α), 0.96 (3H, t, J_{3,3’}=7.4, H-3’), 0.94 (3H, s, H-19). ^13C NMR (125 MHz, CDCl_3): δ 209.4, 173.8, 143.0, 122.1, 63.0, 62.1, 55.1, 51.8, 49.8, 47.5, 46.1, 44.4, 40.3, 38.5, 37.4, 37.3, 32.4, 31.9, 31.7, 28.8, 22.1, 11.9.

(1S, 2S, 4S, 5S, 6R, 12R)-5-Hydroxy-4,8,8-trimethyl-16-propyl-16-azapentacyclo [10.3.1.0^2,0^6,12]hexadec-10-ene-14-one (5) was obtained as a yellow oil, [a]_D^20 = +62° (c 1.00, CHCl_3). EI-MS, m/z (% rel. int.): 329.2341 (M^+), 100, C_21H_17NO_2 requires 329.2341, 314 (37), 286 (100), 272 (70), 258 (63), 244 (25), 216 (29), 28 (25). ^1H NMR (500 MHz, CDCl_3): δ 3.66 (1H, m, H-12), 3.26 (1H, d, J_{5-6}=9.5, H-5), 3.10 (1H, m, H-3), 2.74 (1H, dd, J_{13b-13a}=4.8, J_{13b-13a}=13, H-13β), 2.71 (1H, dd, J_{15b-15a}=4.2, J_{15u-15b}=12.8, H-15α), 2.37 (1H, m, H-15β), 2.34 (1H, m, H-13α), 2.27 (3H, m, H-1’ and H-6), 1.97 (2H, m, H-9), 1.88 (1H, dd, J_{6b-6a}=8.5, J_{7b-7a}=12.4, H-7β), 1.50 (2H, sext, J_{3,3’}=7.5, H-2’), 1.30–1.25 (2H, m, H-1α and H-7α), 1.22 (3H, s, H-18), 0.98 (6H, s, H-19 and H-17), 0.92 (3H, t, J_{5,5’}=7.4, H-3’), 0.23 (1H, d, J_{15b-15a}=5.2, H-1β). ^13C NMR (125 MHz, CDCl_3): δ 209.5 (C-14), 134.4 (C-11), 130.2 (C-10), 78.2 (C-5), 62.5 (C-12), 59.6 (C-3), 54.7 (C-1’), 48.2 (C-15), 47.5 (C-13), 45.5 (C-7), 44.4 (C-6), 44.4 (C-9), 38.3 (C-8), 36.4 (C-2), 28.9 (C-17 or C-19), 28.6 (C-4), 28.5 (C-19 or C-17), 21.8 (C-2’), 18.6 (C-18), 17.6 (C-1), 11.9 (C-3).

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References

1. Article No. 1 in a series on ‘Novel Bioactive Compounds from Reactive Fungal Metabolites’.

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