Synthesis of novel spiro-isoxazoline and spiro-isoxazolidine derivatives of tomentosin†
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A series of novel enantiomerically pure spiro-isoxazolines and spiro-isoxazolidines were synthesized regioselectively by 1,3-dipolar cycloaddition using respectively two dipoles, nitrones and nitrile oxides, on the exocyclic double bond of the B ring of tomentosin (α-methylene-γ-butyrolactone), a sesquiterpene lactone extracted from Dittrichia viscosa.

Introduction

Plants have a long history as therapeutics in the treatment of human diseases and have been a continuous source of inspiration for the development of new medicines. Among them, the genus *Inula* (Asteraceae) comprises more than 100 species, many of which are widely used in traditional medicine for a variety of biological purposes including anti-inflammatory, anti-cancer and antibacterial activities.1-3 Numerous compounds of interest have been isolated and identified from these plants such as flavonoids, monoterpenes, triterpenoids, and polyphenols. This genus is also a rich source of sesquiterpene acids and lactones. Many studies have focused on sesquiterpene lactones since they exhibit a wide range of biological properties4-6 and have candidates in different phases of clinical trials such as parthenolide, costunolide, helenalin, and artemisinin (Fig. 1). The cytotoxicity of sesquiterpene lactones was partly attributed to the presence of potential alkylating agents such as the α-methylene-γ-lactone moiety, which are prone to covalently react with biological nucleophiles, e.g., l-cysteine, in a Michael-type addition.7-12 This highly electrophilic structure may also be the origin of a major contact allergen effect and plants that contain sesquiterpene lactones are held responsible for an increasing number of cases of contact dermatitis.13-15

Using this reactive site, various structural modifications have been carried out to obtain less toxic and more reactive candidates and lately the introduction of spiro-heterocyclic molecular frameworks has aroused particular interest among medicinal chemists.16,17 For example, the Ding and Kumar groups18-20 recently synthesized spiro-isoxazoline and spiro-isoxazolidine derivatives of parthenin, α-santonin and artemisinin and promising anti-cancer activities were obtained. As part of the Moroccan plant development program,21-25 Dittrichia viscosa L. Greuter, an invasive perennial weed, was particularly examined.26,27 This plant is used either as extracts or essential oil in traditional Moroccan medicine for its antipyretic, anti-septic and anti-inflammatory properties.28,29 Easily accessible, it is a renewable source of sesquiterpene lactones such as tomentosin (1),30,31 also known as xanthalongin. This molecule is straightforwardly isolated in respectively 1.5% with respect to the dry weight of the aerial part of the plant (Fig. 1).21,22,23 Tomentosin was already reported to act as a cytotoxic and anti-inflammatory agent34-36 but despite this biological potential, it has received little pharmacological attention so far. Therefore, we propose herein the introduction of an isoxazoline and an isoxazolidine functionality to form libraries of structurally original spiro-bicyclic analogues of tomentosin by 1,3-dipolar cycloaddition with two dipoles, nitrones and nitrile oxides on the exocyclic double bond of the B ring of tomentosin (Fig. 2).

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Results and discussion

The spiroisoxazoline derivatives of tomentosin were synthesized through a 1,3-dipolar cycloaddition of various aldoximes with the exocyclic ring B double bond (Scheme 1).

Nitrile oxides were prepared by converting various aromatic aldehydes to the corresponding oximes via the reaction with hypochlorite anion present in bleach. The bleach was used in two steps: initially to produce chlorooxime and then thanks to its basicity to induce dehydrohalogenation, leading to the nitrile oxide. Experimentally, aldoxime was mixed with the tomentosin in THF, and then a bleach solution (14.5% of chlorine) was added dropwise during 12 hours. Only one diastereoisomer was isolated and characterized by NMR spectroscopic analysis and mass spectrometry. The $^1$H NMR spectra of the spiroisoxazoline derivatives of tomentosin $3a$ showed the disappearance of the alkene protons along with the appearance of two doublets at respectively 3.42 and 3.58 ppm that confirm the selectivity of the nitrile oxide cycloaddition. The reaction took place whatever the substituent in the $para$ position of the aryl entity, whether electron donating ($\text{CH}_3$, OCH$_3$) or electron-attracting (F). When a poor or electron-rich heteroaryl was used, the reaction did not take place and only the starting material was recovered.

The spiroisoxazolidine derivatives of tomentosin were obtained through 1,3-dipolar cycloaddition of various nitrones 4 in refluxing dry benzene (Scheme 2). Nitrones 4 were straightforwardly obtained according to the literature procedure in which nitroaryl was first reduced in the presence of zinc and acetic acid to obtain the corresponding aryl hydroxylamines that were condensed with various aromatic aldehydes.

The spiro-isoxazolidines 5 were obtained as one diastereomer after purification by flash chromatography. The operating conditions are compatible with the introduction of nitrones with aryl entities bearing electron-donating ($\text{CH}_3$, OCH$_3$) or electron-attracting ($\text{CF}_3$, F) substituents. The use of nitrones with heteroaryl entities was carried out successfully. It should be noted that the use of toluene instead of benzene, for environmental reasons, did not unfortunately allow us to obtain the products. The structures of the spiroisoxazolidines were confirmed by their $^1$H, $^13$C and 2 D NMR spectroscopic data as described for 5q (Fig. 3). The $^1$H and $^1$H-COSY data showed the correlation of H-7 with H-8 and H-8 with H-16. Further, the HMBC experiment showed the correlation of H-7 and H-8 with C-17 and H-7 with C-16 (Fig. 3).

In the $^1$H NMR comparison with the literature data, Reddy et al. obtained two diastereomers. Each diastereomer was isolated and the clear chemical shift deviation of the benzylic proton adjacent to the nitrogen atom in the isoxazolidine ring.
between two diastereomers was observed in $^1$H NMR. In the major isomer this proton appeared at 5 ppm, but in the minor isomer this signal shifted toward a more shielding region and appeared approximately at 4 ppm.

## Experimental section

### General information

All reagents were purchased from commercial suppliers and were used without further purification. The reactions were monitored by thin-layer chromatography (TLC) analysis using silica gel (60 F254) plates. Compounds were visualized by UV irradiation. Flash column chromatography was performed on silica gel 60 (230–400 mesh, 0.040–0.063 mm). $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AVANCE II spectrometer at 250 MHz ($^{13}$C, 62.9 MHz) and on a Bruker AVANCE III HD spectrometer at 400 MHz, CDCl$_3$).$^1$H NMR (400 MHz, CDCl$_3$) δ 7.55 (d, J = 8.0 Hz, 2H), 7.21 (d, J = 7.9 Hz, 2H), 5.43 (dd, J = 9.4, 3.5 Hz, 1H), 4.90 (ddd, J = 11.4, 6.5, 4.6 Hz, 1H), 3.56 (d, J = 16.8 Hz, 1H), 3.40 (d, J = 17.0 Hz, 1H), 2.84 (ddd, J = 13.0, 6.6, 3.0 Hz, 1H), 2.59–2.39 (3H, 3H), 2.38 (s, 3H), 2.36–2.15 (m, 4H), 2.13 (s, 3H), 2.02–1.86 (m, 2H), 1.16 (d, J = 7.0 Hz, 3H);$^{13}$C NMR (101 MHz, CDCl$_3$) δ 208.0 (C), 173.7 (C), 156.1 (C), 145.1 (C), 141.1 (C), 129.6 (2 CH), 127.0 (2 CH), 125.7 (C), 120.7 (CH), 88.8 (C), 80.1 (C), 46.2 (CH), 42.6 (CH$_2$), 37.7 (CH$_2$), 36.6 (CH$_2$), 33.1 (CH), 30.8 (CH$_3$), 30.1 (CH$_3$), 22.7 (CH$_3$), 21.6 (CH$_3$), 21.2 (CH$_2$); HRMS (ESI$^+$): calc. For C$_{22}$H$_{26}$NO$_5$ [M + H]$^+$ 381.1856; found 381.1856.

### General procedure for the synthesis of spiro-isoxazolines

The appropriate nitronal [1.1 equiv.] was added to a solution of tomentosin (1 equiv.) in benzene (2 mL). The resulting suspension was heated to reflux for 12 h. Then the reaction mixture was concentrated under reduced pressure and the}
crude material was purified by flash chromatography on silica gel to provide the expected spiro-isoxasolidine.

13-[Phenylamine], phenyl-methyl)-11, N-epoxy-tomentosin (5a). Following the general procedure, using tomentosin (80 mg, 0.32 mmol) and corresponding nitrone (70 mg, 0.35 mmol), column chromatography on silica gel (petroleum ether/ethyl acetate 6/4) provided 5a (104 mg, 0.23 mmol, 72%) as a yellowish oil; [α]D -180.0 +58.6 (c 1.0, CH2Cl2); 1H NMR (400 MHz, CDCl3) δ 7.49 (d, J = 7.8 Hz, 2H), 7.41–7.35 (m, 2H), 7.34–7.28 (m, 1H), 7.21–7.12 (m, 2H), 6.96–6.86 (m, 3H), 5.44–5.37 (m, 1H), 5.14–5.07 (m, 1H), 4.96–4.89 (m, 1H), 2.85 (td, J = 12.3, 11.0, 5.4 Hz, 2H), 2.60–2.17 (m, 7H), 2.15 (s, 3H), 2.13–2.07 (m, 1H), 1.92 (dd, J = 19.2, 12.8, 7.4 Hz, 2H), 1.14 (d, J = 6.9 Hz, 3H); 13C NMR (101 MHz, CDCl3) δ 208.2 (C), 174.9 (C), 151.4 (C), 145.0 (C), 140.7 (C), 129.3 (2 CH), 128.9 (2 CH), 128.2 (CH), 126.9 (2 CH), 122.8 (CH1), 121.4 (CH), 115.8 (2 CH), 85.6 (C), 80.0 (C), 70.4 (CH), 46.0 (CH), 43.9 (CH), 42.9 (CH3), 36.9 (CH3), 33.4 (CH), 31.0 (CH3), 30.3 (CH3), 23.1 (CH1), 21.4 (CH1); HRMS (ESI)−: calc. For C29H33NO4 [M + H]+ 454.2325; found 454.2325.

13-[Phenylamine], (p-tolyl)-methyl-11, N-epoxy-tomentosin (5b). Following the general procedure, using tomentosin (80 mg, 0.32 mmol) and corresponding nitrone (74 mg, 0.35 mmol) column chromatography on silica gel (petroleum ether/ethyl acetate 6/4) provided 5b (104 mg, 0.22 mmol, 69%) as a yellowish oil; [α]D -180.0 -59.7 (c 1.0, CH2Cl2); 1H NMR (400 MHz, CDCl3) δ 7.37 (d, J = 7.7 Hz, 2H), 7.17 (dd, J = 13.8, 6.8, 2.7 Hz, 4H), 6.96–6.87 (m, 3H), 5.42 (dd, J = 9.4, 3.5 Hz, 1H), 5.06 (dd, J = 9.5, 6.7 Hz, 1H), 4.91 (dd, J = 11.4, 6.5, 4.4 Hz, 1H), 2.91–2.76 (m, 2H), 2.60–2.37 (m, 4H), 2.36 (s, 3H), 2.34–2.17 (m, 3H), 2.15 (s, 3H), 2.13–2.06 (m, 1H), 1.93 (dt, J = 14.5, 11.5 Hz, 2H), 1.14 (d, J = 6.9 Hz, 3H); 13C NMR (101 MHz, CDCl3) δ 208.0 (C), 174.8 (C), 151.3 (C), 144.7 (C), 137.8 (C), 137.4 (C), 129.8 (2 CH), 128.7 (2 CH), 126.7 (2 CH), 122.6 (2 CH), 121.1 (CH), 115.7 (2 CH), 85.3 (C), 79.7 (C), 70.1 (CH), 45.8 (CH), 43.8 (CH2), 42.7 (CH3), 36.7 (CH3), 33.2 (CH), 30.8 (CH3), 30.1 (CH3), 22.9 (CH2), 21.2 (CH2); HRMS (ESI)−: calc. For C29H33NO4 [M + H]+ 464.2428; found 464.2428.
Following the general procedure, using tomotensin (80 mg, 0.32 mmol) and corresponding nitrone (89 mg, 0.35 mmol) column chromatography on silica gel (petroleum ether/ethyl acetate 3/7) provided 5g (78 mg, 0.16 mmol, 50%) as an orange oil; [α]D 20 +68.0 (c 1.0, CH2Cl2); 1H NMR (400 MHz, CDCl3) δ 7.21–7.14 (m, 2H), 7.01 (d, J = 1.9 Hz, 1H), 6.98–6.89 (m, 5H), 5.67 (s, 1H), 5.45–5.39 (m, 1H), 5.03 (dd, J = 9.6, 6.5 Hz, 1H), 4.91 (dd, J = 11.3, 6.7, 4.3 Hz, 1H), 3.88 (s, 3H), 2.87–2.77 (m, 2H), 2.60–2.17 (m, 7H), 2.14 (s, 3H), 2.10 (dt, J = 13.7, 4.1 Hz, 1H), 1.98–1.86 (m, 2H), 1.14 (d, J = 6.9 Hz, 3H); 13C NMR (101 MHz, CDCl3) δ 208.0 (C), 174.9 (C), 162.5 (d, J = 246.4 Hz, C), 148.3 (C), 144.8 (C), 135.7 (d, J = 3.2 Hz, C), 134.0 (C), 129.4 (2 CH), 128.6 (d, J = 8.1 Hz, 2 CH), 121.1 (CH), 116.9 (2 CH), 115.9 (d, J = 21.5 Hz, 2 CH), 85.2 (C), 79.8 (C), 70.0 (CH), 46.0 (CH), 43.6 (CH3), 42.7 (CH3), 36.7 (CH3), 33.2 (CH3), 30.8 (CH3), 22.9 (CH3), 21.2 (CH3); HRMS (ESI−): calc. For C23H23NO3 [M + H]+ 478.2387; found 478.2388.

13-[p-Tolylamine), (4-trifluoromethylphenyl)-methyl]-11, N-epoxy-tomentosin (5k). Following the general procedure, using tomotensin (80 mg, 0.32 mmol) and corresponding nitrone (98 mg, 0.35 mmol) column chromatography on silica gel (petroleum ether/ethyl acetate 5/5) provided 5k (104 mg, 0.20 mmol, 62%) as a yellow oil; [α]D 20 +98.7 (c 1.0, CH2Cl2); 1H NMR (400 MHz, CDCl3) δ 7.63 (t, J = 6.1 Hz, 4H), 7.00 (d, J = 8.1 Hz, 2H), 6.83 (d, J = 8.1 Hz, 2H), 5.40 (dd, J = 9.5, 3.4 Hz, 1H), 5.13 (d, J = 9.4, 6.7 Hz, 1H), 4.91 (dd, J = 11.3, 6.7, 4.5 Hz, 1H), 2.84 (dd, J = 12.6, 6.6 Hz, 2H), 2.59–2.25 (m, 7H), 2.24 (s, 3H), 2.15 (s, 3H), 2.13–2.06 (m, 1H), 1.91 (dd, J = 13.7, 11.3 Hz, 2H), 1.14 (d, J = 6.9 Hz, 3H); 13C NMR (101 MHz, CDCl3) δ 208.0 (C), 174.7 (C), 148.3 (C), 144.9 (C), 144.5 (C), 133.0 (C), 130.3 (d, J = 32.5 Hz, C), 129.5 (2 CH), 127.2 (2 CH), 126.0 (q, J = 3.7 Hz, 2 CH), 124.1 (d, J = 272.1 Hz, C), 121.0 (CH), 116.5 (2 CH), 85.4 (C), 79.9 (C), 69.9 (CH), 45.9 (CH), 43.5 (CH), 42.6 (CH), 36.7 (CH3), 33.2 (CH3), 30.8 (CH3), 22.9 (CH3), 21.2 (CH3), 20.8 (CH3); HRMS (ESI−): calc. For C21H18F2NO4 [M + H]+ 528.2355; found 528.2356.

13-[4-Fluorophenylamine), phenyl-methyl]-11, N-epoxy-tomentosin (5l). Following the general procedure, using tomotensin (80 mg, 0.32 mmol) and corresponding nitrone (95 mg, 0.20 mmol) column chromatography on silica gel (petroleum ether/ethyl acetate 6/4) provided 5l (105 mg, 0.23 mmol, 72%) as a yellowish oil; [α]D 20 +55.8 (c 1.0, CH2Cl2); 1H NMR (400 MHz, CDCl3) δ 7.57 (d, J = 8.4 Hz, 2H), 6.97 (d, J = 8.2 Hz, 2H), 6.88 (d, J = 8.2 Hz, 2H), 5.42 (dd, J = 9.4, 3.5 Hz, 1H), 5.02 (dd, J = 9.6, 6.4 Hz, 1H), 4.89 (dd, J = 11.4, 6.8, 4.4 Hz, 1H), 2.85–2.78 (m, 2H), 2.60–2.25 (m, 7H), 2.23 (s, 3H), 2.15 (s, 3H), 2.10 (dt, J = 13.8, 4.1 Hz, 1H), 1.98–1.88 (m, 2H), 1.14 (d, J = 6.9 Hz, 3H); 13C NMR (101 MHz, CDCl3) δ 208.0 (C), 171.5 (C), 159.3 (d, J = 241.6 Hz, C), 147.0 (d, J = 2.5 Hz, C), 144.8 (C), 139.5 (C), 129.1 (2 CH), 128.3 (CH), 127.0 (2 CH), 120.9 (CH), 118.6 (d, J = 8.0 Hz, 2 CH), 115.4 (d, J = 22.6 Hz, 2 CH), 85.1 (C), 79.7 (CH), 71.1 (CH), 45.7 (CH), 43.9 (CH3), 42.7 (CH3), 36.7 (CH3), 33.5 (CH3), 30.8 (CH3), 30.1 (CH3), 22.8 (CH3), 21.2 (CH3), 20.8 (CH3); HRMS (ESI−): calc. For C23H19FNO4 [M + H]+ 528.2355; found 528.2356.
(dd, J = 9.9, 6.3 Hz, 1H), 4.85 (ddd, J = 11.4, 7.0, 4.1 Hz, 1H), 2.90–2.75 (m, 2H), 2.60–2.40 (m, 5H), 2.35 (s, 3H), 2.32–2.19 (m, 2H), 2.15 (s, 3H), 2.10 (dt, J = 13.7, 4.2 Hz, 1H), 2.02–1.85 (m, 2H), 1.14 (d, J = 6.9 Hz, 3H); 13C NMR (101 MHz, CDCl3) δ 174.6 (C), 174.3 (C), 150.8 (C), 144.7 (C), 142.9 (CH), 128.8 (2 CH), 123.4 (CH), 121.4 (CH), 116.5 (2 CH), 110.7 (CH), 108.5 (CH), 85.7 (C), 80.1 (CH), 64.7 (CH), 46.3 (CH2), 42.7 (CH2), 38.5 (CH2), 36.7 (CH2), 33.0 (CH), 30.9 (CH2), 30.1 (CH3), 23.0 (CH2), 21.3 (CH3); HRMS (ESI+): calc. for C26H29NO5 [M + H]+ 436.2118; found 436.2118.

13-[Phenylamino), (thiophen-3-yl)-methyl]-11, N-epoxy-tomentosin (5q). Following the general procedure, using tomentosin (80 mg, 0.32 mmol) and corresponding nitrone (72 mg, 0.35 mmol) column chromatography on silica gel (petroleum ether/ethyl acetate 6/4) provided 5q (99 mg, 0.22 mmol, 68%) as a yellowish oil; [α]D 20 17.0 (c 1.0, CH2Cl2); 13C NMR (101 MHz, CDCl3) δ 208.2 (C), 175.0 (C), 151.2 (C), 145.0 (C), 141.3 (C), 129.0 (2 CH), 127.2 (CH), 126.2 (CH), 123.3 (CH), 122.4 (CH), 121.4 (CH), 116.5 (2 CH), 85.6 (C), 80.1 (CH), 67.0 (CH), 46.3 (CH2), 42.9 (CH2), 42.4 (CH2), 36.9 (CH2), 33.4 (CH), 31.0 (CH2), 30.3 (CH3), 23.2 (CH2), 21.4 (CH3); HRMS (ESI+): calc. for C28H30NO5S [M + H]+ 452.1887; found 452.1890.

Conclusions

In summary, we described here the synthesis of interesting spiro-isoxazolidine and isoxazoline derivatives of tomentosin by a 1,3-dipolar cycloaddition of respectively nitrones and nitrile oxides to the natural compound. We used an enantiomerically pure and natural starting material, thereby limiting the chemical impact on the environment. This procedure allowed us to generate enantiomerically pure spiro compounds in one dia stereoisomer form with a limited number of steps.

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