Xanthane sesquiterpenoids from the roots and flowers of Xanthium cavanillesii

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Abstract

The sesquiterpene lactones xanthanodiene, 4-epi-xanthanol, 4-epi-isoxanthanol, and 4-epi-xanthinin, as well as the xanthanolide derivative 4-oxo-bedfordia acid were isolated from the chloroform extracts of roots and flowers of Xanthium cavanillesii Schouw. The identities of these compounds were corroborated through comparison of their spectroscopic data, including IR, MS, and \textsuperscript{1}H and \textsuperscript{13}C NMR assignments, with literature reports. In addition, the structural characterization of 4-oxo-bedfordia acid was revisited and a comprehensive spectroscopic study of the compound is presented. This is to our knowledge the first phytochemical investigation of the roots of X. cavanillesii, and of flowers in the whole Xanthium genus.

Keywords: Abrojo; 4-Oxo-bedfordia acid; Xanthane sesquiterpenoids; Xanthanolides; Xanthium cavanillesii Schouw.

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Experimental

General experimental procedures
TLC was performed on silica gel plates (Macherey Nagel, Dürin, Germany) using CH$_2$Cl$_2$-acetone as solvent and H$_2$SO$_4$/Δ or p-hydroxybenzaldehyde as detection reagents. Column chromatography was carried out on 10-40 mesh silica gel H (Sigma-Aldrich, St. Louis, MO, USA). EIMS spectra were recorded on a Shimadzu GCMS-QP5050 single quadrupole mass spectrometer using a 70 eV ionization voltage, while HR-ESIMS and ESIMS$^2$ data were collected on Bruker Daltonics micrOTOF-Q and AB SCIEX 4000 QTrap mass spectrometers, respectively. IR spectra were recorded on Nicolet 8700 or Shimadzu IRAffinity-1 FT-IR spectrophotomers. NMR experiments were performed on Bruker AVANCE 400, AVANCE III 400, or AVANCE III 500 spectrometers operating at $^1$H frequencies of 400.13 and 500.13 MHz, and $^{13}$C frequencies of 100.62 and 125.76 MHz, respectively, using tetramethylsilane (TMS) as internal standard. Resonance assignments were in many cases corroborated with 1D-TOCSY data obtained at varying mixing times (120, 80, and 40 ms), and $^1$H homonuclear decoupling experiments were employed to determine coupling constants in complex multiplets. 1D-NOESY experiments were carried out using the DPFGSE-NOE pulse sequence and a mixing time of 300 ms (Stott et al. 1997). Gradient-enhanced COSY, HSQC, and HMBC spectra were acquired using standard pulse sequences provided with the instruments. Multiplet and coupling constant analysis were performed using the routines available in MNova 10.0 (Mestrelabs Research, S. L., Santiago de Compostela, Spain).

Plant material
X. cavanillesii was collected near Solymar, Canelones department, Uruguay (S 34° 49’ 35” W 55° 57’ 54”). A voucher specimen was authenticated by Prof. Eduardo Alonso Paz and deposited in the herbarium of the Cátedra de Botánica, Facultad de Química, UdelaR, with ID number 3510 MVFQ.

Extraction and Isolation
Fresh X. cavanillesii roots (22 g) were coarsely chopped and extracted by maceration in CH$_2$Cl$_2$ (250 mL) at room temperature in the dark for 72 hours. The solids were filtered, the solvent was evaporated under vacuum, and the resulting extract (1.2 g) was submitted to silica gel column chromatography using CH$_2$Cl$_2$ as eluent. Fractions 5 to 17 from this first column were pooled and resubmitted to chromatographic separation using a CH$_2$Cl$_2$-AcOEt gradient (100:0 to 90:10, v/v). Fractions 44-65 from this second column were pooled, loaded onto a preparative silica gel TLC plate, and developed with CH$_2$Cl$_2$-acetone (9:1) as eluent to yield compound 1 (6 mg, $R_f = 0.65$).
Fresh flowers of *X. cavanillesii* (9.2 g) were extracted by maceration in CH$_2$Cl$_2$ (150 mL) at room temperature in the dark for 72 hours. The solids were filtered, the solvent was evaporated under vacuum, and the resulting extract (0.82 g) was submitted to silica gel column chromatography using CH$_2$Cl$_2$-acetone (90:10) as eluent. Fractions 15-17, 37-50, and 74-99 from this column were pooled, loaded onto preparative silica gel TLC plates, and developed with CH$_2$Cl$_2$-acetone (6:1) as eluent to yield compounds 2 (7 mg, $R_f = 0.32$), 3 (5 mg, $R_f = 0.39$), 4 (6 mg, $R_f = 0.52$), and 5 (16 mg, $R_f = 0.36$).

**Xanthanodiene (1)**

Yellow oil. IR (thin film) cm$^{-1}$: 3028, 1770. EIMS m/z: 232, 217, 199. HR-ESIMS m/z: 255.1350 [M+Na]$^+$ (calcld. for C$_{15}$H$_{20}$O$_2$Na: 255.1361). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 6.25 (1H, d, $J = 2.4$, 0.6, H13), 5.61 (1H, dd, $J = 2.2$, 0.6, H13'), 5.50 (1H, dd, $J = 3.6$, 3.2, H1), 4.55 (1H, ddd, $J = 9.4$, 7.6, 7.4, H8), 2.93 (1H, ddddd, $J = 12.0$, 7.4, 5.4, 2.3, 2.2, H7), 2.61 (1H, dd, $J = 12.6$, 7.6, H9), 2.34 (1H, dd, $J = 12.6$, 9.4, H9'), 2.05 (2H, ABXY, H2 & H2'), 1.74 (1H, $J = 14.2$, 12.0, H6), 1.64 (1H, dd, $J = 14.2$, 5.4, H6'), 1.61 (1H, dd, $J = 14.2$, 11.8, H4), 1.55 (2H, ABXY, H3 & H3'), 0.95 (3H, d, $J = 6.5$, H14), 0.94 (3H, s, H15). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$: 170.7 (C12), 139.4 (C11), 138.4 (C10), 126.1 (C5), 117.6 (C13), 82.6 (C8), 78 (C2), 63.6 (C4), 47.3 (C7), 42.7 (C3), 36.5 (C9), 29.7 (C10), 24.6 (C6), 22.6 (C15), 19.8 (CH$_3$CO), 18.3 (C14).

**4-epi-Xanthanol (2)**

Colourless oil. IR (thin film) cm$^{-1}$: 3350, 1762, 1735. EIMS m/z: 248, 230, 204, 189, 176. HR-ESIMS m/z: 331.1557 [M+Na]$^+$ (calcld. for C$_{17}$H$_{24}$O$_5$Na: 331.1521). $^1$H NMR (400 MHz, CD$_3$OD): $\delta$ 6.11 (1H, d, $J = 3.4$, H13), 5.96 (1H, dd, $J = 9.3$, 3.5, H5), 5.59 (1H, d, $J = 3.2$, H13'), 5.31 (1H, dd, $J = 9.7$, 4.1, H2), 4.39 (1H, ddd, $J = 12.4$, 10.4, 2.9, H8), 3.76 (1H, ddd, $J = 9.0$, 6.3, 3.8, H4), 2.84 (1H, ddq, $J = 4.6$, 3.6, 7.4, H10), 2.63 (1H, ddd, $J = 15.9$, 9.3, 2.3, H6), 2.49 (1H, ddddd, $J = 11.2$, 10.4, 3.4, 3.2, 2.3, H7), 2.28 (1H, ddd, $J = 12.5$, 4.6, 2.9, H9), 2.15 (1H, ddd, $J = 15.9$, 11.2, 3.5, H6'), 2.06 (3H, s, CH$_3$CO), 1.83 (1H, ddd, $J = 13.9$, 9.7, 3.8, H3), 1.77 (1H, ddd, $J = 12.5$, 12.4, 3.6, H9'), 1.64 (1H, ddd, $J = 13.9$, 9.0, 4.1, H3'), 1.20 (3H, d, $J = 6.3$, H15), 1.15 (3H, d, $J = 7.4$, H14). $^{13}$C NMR (100 MHz, CD$_3$OD): $\delta$: 170.8 (CH$_3$CO), 170.6 (C12), 146 (C1), 139.9 (C11), 126.1 (C5), 117.6 (C13), 82.6 (C8), 78 (C2), 63.6 (C4), 47.3 (C7), 42.7 (C3), 36.5 (C9), 29.7 (C10), 24.6 (C6), 22.6 (C15), 19.8 (CH$_3$CO), 18.3 (C14).

**4-epi-Isoxanthanol (3).**

Pale yellow oil. IR (thin film) cm$^{-1}$: 3400, 1765,1740. EIMS m/z: 248, 230, 204, 189, 176. HR-ESIMS m/z: 331.1557 [M+Na]$^+$ (calcld. for C$_{17}$H$_{24}$O$_5$Na: 331.1521). $^1$H NMR (400 MHz, CD$_3$OD):
δ 6.11 (1H, d, J = 3.4, H13), 5.85 (1H, dd, J = 9.3, 3.4, H5), 5.59 (1H, d, J = 3.1, H13’), 5.00 (1H, ddq, J = 7.8, 5.2, 6.4, H4), 4.41 (1H, ddd, J = 12.6, 10.4, 3.0, H8), 4.03 (1H, dd, J = 8.4, 5.2, H2), 2.81 (1H, ddq, J = 4.5, 3.6, 7.4, H10), 2.60 (1H, ddd, J = 15.8, 9.3, 2.3, H6), 2.51 (1H, ddddd, J = 11.3, 10.4, 3.4, 2.3, H7), 2.30 (1H, ddd, J = 12.4, 4.5, 3.0, H9), 2.16 (1H, ddd, J = 15.8, 11.3, 3.4, H6’), 2.04 (3H, s, CH₃CO), 1.78 (1H, ddd, J = 12.6, 12.4, 3.6, H9’), 1.73 (2H, ddd, J = 13.9, 7.8, 5.2, H3), 1.71 (1H, ddd, J = 13.9, 8.4, 5.2, H3’), 1.25 (3H, d, J = 6.4, H15), 1.22 (3H, d, J = 7.4, H14). ¹³C NMR (100 MHz, CD₃OD): δ 171.1 (CH₃C=O), 170.7 (C12), 149.7 (C1), 140 (C11), 123.3 (C5), 117.6 (C13), 82.9 (C8), 74.8 (C2), 68.5 (C4), 48.1 (C7), 41.5 (C3), 36.4 (C9), 29.1 (C10), 24.6 (C6), 20.5 (CH₃CO), 19.8 (C15), 19.1 (C14).

2-epi-Xanthin (4).
Pale yellow oil. IR (thin film) cm⁻¹: 3400, 1765, 1740. EI-MS m/z: 246, 231, 204, 187, 122. HR-ESIMS m/z 329.1346 [M+Na]⁺ (calcld. for C₁₇H₂₁O₅Na: 329.1365). ¹H NMR (400 MHz, CDCl₃): δ 6.19 (1H, d, J = 3.3, H13), 6.00 (1H, dd, J = 9.3, 3.5, H5), 5.61 (1H, dd, J = 8.2, 5.6, H2), 5.47 (1H, d, J = 3.1, H13’), 4.30 (1H, ddd, J = 12.4, 9.6, 3.0, H8), 2.94 (1H, dd, J = 16.0, 8.2, H3), 2.79 (1H, ddq, J = 4.4, 3.6, 7.3, H10), 2.62 (1H, dd, J = 16.0, 5.6, H3’), 2.56 (1H, ddd, J = 16.2, 9.3, 2.6, H6), 2.43 (1H, ddddd, J = 11.3, 9.6, 3.3, 3.1, 2.6, H7), 2.35 (1H, ddd, J = 12.6, 4.4, 3.0, H9), 2.20 (3H, s, H15), 2.12 (1H, ddd, J = 16.2, 11.3, 3.5, H6’), 2.06 (3H, s, CH₃CO), 1.74 (1H, ddd, J = 12.6, 12.4, 3.6, H9’), 1.13 (3H, d, J = 7.3, H14). ¹³C NMR (100 MHz, CDCl₃): δ 169.9 (C12), 169.8 (CH₃CO), 144.7 (C1), 139.2 (C11), 127.2 (C5), 118.7 (C13), 82 (C8), 76 (C2), 47.9 (C7), 47.2 (C3), 36.8 (C9), 30.5 (C15), 29.8 (C10), 25.3 (C6), 21.1 (CH₃CO), 19.3 (C14).

4-oxo-Bedfordia acid (5).
Pale yellow waxy solid. IR (ATR) cm⁻¹: 2926, 1714, 1694, 1624, 1161. ESIMS² (249→): m/z 231, 205, 178, 147, 135. HR-ESIMS m/z: 273.1812 [M+Na]⁺ (calcld. for C₁₃H₂₂O₃Na: 273.1467). ¹H NMR and ¹³C NMR: see Table S1.

References
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Table S1. $^1$H (500 MHz) and $^{13}$C NMR (100 MHz) data for compound 5 in CDCl$_3$ ($\delta$ in ppm, $J$ in Hz).

| Position | $\delta_H$       | $\delta_C$ |
|----------|------------------|------------|
| 1        | -                | 147.3      |
| 2        | 2.25 (ABXY)      | 33.9       |
| 3        | 2.53 (ABXY)      | 42.7       |
| 4        | -                | 208.9      |
| 5        | 5.42 (dd, $J$ = 9.3, 4.0) | 122.6 |
| 6        | 2.07 (ddd, $J$ = 14.9, 9.3, 1.8) | 33.4 |
|          | 2.20 (ddd, $J$ = 14.9, 4.0, 11.4) |   |
| 7        | 2.50 (dddddd, $J$ = 11.4, 6.8, 5.2, 1.8, 1.0) | 39.5 |
| 8        | 1.80 (ABMXY)     | 31.5       |
| 9        | 1.62 (dddd, $J$ = 13.7, 10.1, 5.3, 3.4) | 32.6 |
|          | 1.69 (dddd, $J$ = 13.7, 5.3, 5.1, 3.2) |   |
| 10       | 2.30 (ddq, $J$ = 5.1, 3.4, 7.3) | 37.1 |
| 11       | -                | 146.1      |
| 12       | 5.64 (dd, $J$ = 1.0, 0.9) | 124.7 |
|          | 6.28 (d, $J$ = 0.9) |   |
| 13       | -                | 171.7      |
| 14       | 1.11 (d, $J$ = 7.3) | 16.3 |
| 15       | 2.16 (s)         | 30.0       |
| CO$_2$H  | 10.94 (bs)       | -          |