Polyhydroxyserratane triterpenoids from *Diphasiastrum complanatum*

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Abstract

Serratane triterpenoids were identified from *Diphasiastrum complanatum* (L.) Holub, including serratane-3α,14α,15α,20β,21β,24,29-heptol (1), 3α,20β,21β-trihydroxserrat-14-en-24-oic acid (2), 3β,20β,21β-trihydroxserrat-14-en-24-oic acid (3), 3α,20β,21β-trihydroxy-16-oxoserrat-14-en-24-oic acid (4), and 16-oxolycanitin-29-yl E-4′-hydroxyl-3′-methoxycinnamate (5) on the basis of their spectroscopic data as well as nine known analogs.

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Keywords: *Diphasiastrum complanatum*; Lycopodiaceae; Serratane triterpenoid; Polyhydroxylated derivatives

1. Introduction

Serratane triterpenoids were first isolated from *Lycopodium clavatum* L. growing in Japan by Inubushi Y. et al. in 1962. *Lycopodium* species contain lycopodium alkaloids (Ma and Gang, 2004) and serratane triterpenoids, some of which possess pharmacological activity (Liu et al., 1986a,b; Tanaka et al., 2003; Houghton et al., 2006). We have previously investigated *Lycopodium japonicum* (Yan et al., 2005a,b) and *Phlegmariurus squarrosus*. *Diphasiastrum complanatum* (L.) is distributed in Sichuang, Yunnan, Guizhou, Tibet Provinces and is used as a traditional Chinese herbal medicine for treatment of arthritic pain, quadriplegia, and contusion (Jiangsu, 1990). In our search for bioactive metabolites, we investigated the chemical constituents of *D. complanatum* (L.) Holub. We found five new polyhydroxy-derivatives: serratane-3α,14α,15α,20β,21β,24,29-heptol (1), 3α,20β,21β-trihydroxserrat-14-en-24-oic acid (2), 3β,20β,21β-trihydroxserrat-14-en-24-oic acid (3), 3α,20β,21β-trihydroxy-16-oxoserrat-14-en-24-oic acid (4), and 16-oxolycanitin-29-yl E-4′-hydroxyl-3′-methoxycinnamate (5), together with nine known compounds, lycoclavanol (6), serratenediol (7), 21-epi-serratenediol (8), lycoclaninol (9), Haruo et al. (1988), wightianol B (10) (Tsuda and Tabata, 1980), lycernuic acid A (11) (Zhang et al., 2002), 16-oxolycanitin-29-yl p-coumarate (12) (Cai and Pan, 1992), lycoclavinin (13) (Tsuda et al., 1975), and α-onocerin (14) (Cai et al., 1989; Pauli, 2000). In this paper, we wish to report the isolation and structure elucidation of five new compounds. Fig. 1

2. Results and discussion

The molecular formula of 1, C_{30}H_{52}O_{7}, was established by negative ESI m/z 523 [M–H]−, and was further confirmed by negative HRESIMS m/z 523.3621 (calcd. for C_{30}H_{51}O_{7}, 523.3634). Analysis of 1H and 13C NMR spectra indicated that 1 was a serratane derivative (Tables 1 and 2). In the 1H and 13C NMR spectra of 1, the expected proton...
and carbon signals for a typical serrate-14-ene double bond were absent and were replaced by two hydroxylated carbons ($\delta_C$ 77.9 (C-14) and 76.8 (CH, C-15)); $\delta_H$ 3.65 dd (H-15)]. The $\alpha$ orientation of these two hydroxy groups was assigned by comparing their carbon shifts with those of lyrchnic acid C (Zhang et al., 2002). Two methyl signals of 1 [\$\delta_C$ 23.4 (C-23) and 23.6 (C-30)] were shifted upfield by 5–6 ppm relative to serratenediol (7) and 21-epi-serratenediol (8) indicating that two CH$_2$O groups were located at C-24 and C-29. In the ROESY, strong correlations of H-24 with Me-25 and Me-28 with H-29 confirmed the positions of the CH$_2$OH groups. The broad resonances of H-3 and H-21 suggested that they were $\beta$ and $\gamma$ positions, respectively. The hydroxyl group at C-20 caused a downfield shift of C-19 to 43.1 ppm. In the ROESY spectrum, the correlations of H-20 with H-29 and Me-28 showed that the C-20 hydroxyl group was $\beta$ position. Thus the structure of diphasiastrol (1) is serratane-3$\alpha$,14$\alpha$,15$\beta$,20$\beta$,21$\beta$,24,29-heptol.

Compound (2) was assigned a molecular formula of C$_{30}$H$_{48}$O$_5$ as deduced from the negative HRFABMS ($m/z$ 487.3403 [M–H$^-\$], calcld. for C$_{30}$H$_{47}$O$_{45}$, 487.3423), in conjunction with the proton and carbon NMR data. The $^{13}$C NMR spectrum of 2 showed characteristic double bonds carbons at $\delta_C$ 70.6 (C-3), 79.6 (C-21), 66.6 (C-20), 6 methyl, 9 methylenes, 4 methines, 5 quaternary carbons, a carbonyl group. Analysis of the $^1$H and $^{13}$C NMR spectra indicated that 2 was related to 3$\alpha$,21$\alpha$-dihydroxyserrat-14-en-24-oic acid (Zhou et al., 2003) apart from ring E. The single peak at $\delta_C$ 3.82 (H-21) indicated that the C-21 OH was $\beta$ orientation (Wang and Lou, 2005). The chemical shift differences of C-19 (+$\Delta$11.1 ppm) and C-21 (+$\Delta$4.0 ppm) relative to 3$\beta$,21$\beta$-dihydroxyserrat-14-en-24-oic acid indicated that there was a hydroxyl group attached to C-20. In the ROESY experiment, correlations of H-20$\alpha$ with H-29, and H-20$\beta$ with H-21$\gamma$ confirmed that the C-20 OH was $\beta$ orientated. On the basis of the above evidence, the chemical structure of 2 was assigned as 3$\alpha$, 20$\beta$, 21$\beta$-trihydroxyserrat-14-en-24-oic acid.

Compound (3) had the same molecular formula as 2, C$_{30}$H$_{48}$O$_5$, established by negative HRFABMS. After carefully analysing $^1$H NMR and $^{13}$C NMR spectra of 3 with those of 2, we concluded that 3 differed from 2 only in the configuration of the hydroxyl group at C-3. The C-3 OH of 3 was assigned a $\beta$-orientation on the basis of its characteristic proton resonance at $\delta_H$ 3.40 (1H, $dd$, $J_1 = 11$ Hz, $J_2 = 4$ Hz, H-3$\gamma$) (Fang et al., 1991). The significant chemical shift value differences of C1–C5 between
Table 2

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<tr>
<td>29</td>
<td>1.34 (s)</td>
<td>0.92 (s)</td>
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<td>0.84 (s)</td>
<td>0.84 (s)</td>
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<td>5.35 (d)/11.0/4.95 (d)/11.0</td>
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<td>1.19 (s)</td>
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<td>1.73 (s)</td>
<td>2.08 (s)</td>
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</table>

*The 1H NMR data for the ester moiety of 5: 6.64 (1H, d, J = 15.0, H-8*), 7.95 (1H, d, J = 15.0, H-7*), 7.35 (1H, d, J = 1.8, H-2*), 7.17 (1H, d, J = 8.0, H-5*), 7.28 (1H, dd, J1 = 8.0, J2 = 1.8, H-6*), 3.75 (3H, s, 3'-OCH3).

3 and 2 further supported the β-orientation of the C-3 OH in 3 (Table 1). Thus the structure of 3 is 3β,20β,21β-trihydroxyserrat-14-en-24-oic acid.

Compound (4) had the molecular formula C30H40O6, established by negative HRFABMS. The 13C NMR spectrum exhibited 30 carbon signals, a ketone group at δC 200.9 (C-16), a carboxyl group δC 180.5 (C-24), a double bond δC 163.7 (C-14) and δC 129.3 (C-15), three oxy methylenes at δC 70.5 (C-3), δC 65.9 (C-20), δC 80.4 (C-21), six methyls, four methines, five quaternary carbons, eight methylenes. Comparison of 4 with 2 showed that they were much similar but differed in the numbers of methylene. There were 9 methylenes in 2, and 8 methylenes and a ketone group in 4, which suggested that one of the methylenes in 2 was oxidized to a ketone group. The position of the ketone group at C-16 was established by the HMBE spectrum and chemical shift changes of C-14 and C-15 (Table 1). Thus compound 4 is unambiguously determined as 3α,20β,21β-trihydroxy-16-oxoserrat-14-en-24-oic acid.

Compound (5) was deduced as C40H56O9 from NMR spectra and confirmed by negative HRFABMS. The 1H and 13C NMR spectra of 5 were almost identical with those of 16-oxocycloactin-29-yl p-coumarate (12) except for the presence of a 1,3,4-trisubstituted aromatic ring at δH 7.35 (1H, d, J = 1.8, H-2*), 7.17 (1H, d, J = 8 Hz, H-5*), 7.28 (1H, dd, J1 = 8 Hz, J2 = 1.8 Hz, H-6* (Siddiqui et al., 1997; Zhu et al., 2002) in place of the p-coumaroyl moiety of 12. The structure was also supported by ion fragments at m/z 503 [M−H−C10H4O5]− (10), 193 [C10H4O4]2+ (75) in the negative FABMS. In the HMBE spectrum, correlations between δH 5.35 (1H, d, J = 11.0 Hz, H-29), 4.95 (1H, d, J = 11.0 Hz, H-29) and δC 43.5 (C-22), 74.3 (C-21), and 167.7 (C-9*) indicated the attachment of the (4′-hydroxyl-3′-methoxy)-E-cinnamate group at C-29. Moreover, HMBE correlations of δC 149.0 (C-3′) with 3.75 (3H, s, OCH3-3′) suggested that the methoxy group was at C-3′ (Siddiqui et al., 1997; Zhu et al., 2002). Hence, 5 was formulated as 16-oxocycloactin-29-yl E-4′-hydroxyl-3′-methoxycinnamate (5) Fig. 2.

3. Conclusion

The structures of five new and nine known serratane triterpenoids from D. complanatum were determined by spectroscopic analysis and comparison with literature data. The heptol (1), diphasiastrol, is the most highly hydroxylated serratane derivative reported thus far.
4. Experimental

4.1. General experimental procedures

Silica gel (200–300 mesh, Qingdao Marine Chemical, China), Lichroprep RP-18 (40–63 μm, Merck, Darmstadt, Germany) and Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd.) were used for column chromatography (CC). Fractions were monitored by TLC, and spots were visualized by heating TLC sprayed with 10% H2SO4.

4.2. Plant material

D. complanatum (L.) Holub. was collected at Jinggu county, Yunnan province, located at 1110 m elevation. A voucher specimen (No. 20041022) has been deposited at the Laboratory of Phytochemistry, Kunming Institute of Botany.

4.3. Extraction and isolation

The powdered material (8.0 kg) was exhaustively extracted with MeOH–H2O (9:1, v/v, 30 L) and then partitioned with EtOAc (1.5 L) to give an EtOAc-soluble fraction (250 g). Part of the EtOAc extract was absorbed on silica gel (200–300 mesh, Qingdao Marine Chemical, China), Lichroprep RP-18 (40–63 μm, Merck, Darmstadt, German) and Sephadex LH-20. Afford four fractions (Fr.) 1 (30 g), 2 (80 g), 3 (50 g), 4 (30 g).

Fr.1 was further submitted to CC (silica gel, CHCl3:MeOH 100:1, 80:1) to give 14 (2 g) and 10 (100 mg). Fr. 2 (80 g) was subjected to CC (silica gel, CHCl3:MeOH 40:1, 20:1) and gave two new fractions and 6 (the main constituent). Fr. 2.1 was further purified by repeated CC (silica gel, CHCl3:MeOH 100:0, 50:1, 30:1) to yield 7 (50 mg) and 8 (20 mg). Repeated CC of Fr. 2.2 gave 4 (50 mg), 9 (30 mg) and 11 (15 mg). Similarly Fr. 3 (silica gel, CHCl3:MeOH 40:1) gave 3 (10 mg) and 5 (15 mg) and a residual fraction which was purified by HPLC (MeOH:H2O 60:40) to obtain 2 (100 mg). Repeated CC of Fr. 4 eluting with CHCl3:MeOH (25:1, 20:1, 15:1) gave 2 sub-fractions and 13 (200 mg). Fr. 4.1, on CC as above with CHCl3:MeOH (20:1) as eluant, yielded 12 (30 mg) and 1 (20 mg), which was purified by Sephadex LH-20.

4.3.1. Serratane-3α,14α,15α,20β,21β,24,29-heptol (1)

White powder, m.p. > 350 °C; [α]D23 +0.64 (MeOH; c 1.12); IR (KBr) νmax 3396 (OH), 2935, 1457, 1389, 1250, 1161, 1038, 982, 672 cm−1; for 13C and 1H NMR spectroscopic data, see Tables 1 and 2; Negative ESIMS m/z (rel. int.): 523 (20), 339 (90), 325 (100), 311 (50); negative HRESIMS: 523.3621 [M+H]+ (calcd. for C30H51O17, 523.3634).

4.3.2. 3α, 20β, 21β-trihydroxyserrat-14-en-24-oic acid (2)

White powder, m.p. 271–273 °C; [α]D23 +15.9 (MeOH; c 1.7); IR (KBr) νmax 3477 (OH), 2969, 2873, 1690 (COOH), 1463, 1387, 1195, 946, 737 cm−1; for 13C and 1H NMR spectroscopic data, see Tables 1 and 2; Negative FABMS m/z (rel. int.): 487 [M+H]+ (100); Negative HRESIMS m/z 487.3403 [M+H]+ (29) (calcd. for C30H47O5, 487.3423).

4.3.3. 3β, 20β, 21β-trihydroxyserrat-14-en-24-oic acid (3)

White powder, m.p. 258–260 °C; [α]D23 +10.7 (MeOH; c 0.81); IR (KBr) νmax 3477 (OH), 2951, 2873, 1690 (COOH), 1463, 1387, 1195, 946, 737 cm−1; for 13C and 1H NMR spectroscopic data, see Tables 1 and 2; Negative FABMS m/z (rel. int.): 487 [M+H]+ (100); Negative HRESIMS m/z 487.3408 [M+H]+ (24) (calcd. for C30H47O5, 487.3423).

4.3.4. 3α, 20β, 21β-trihydroxy-16-oxoserrat-14-en-24-oic acid (4)

White powder, m.p. 276–278 °C; [α]D23 +22.1 (MeOH; c 1.12); IR (KBr) νmax 3444 (OH), 2968, 1710, 1652, 1387, 1231, 1190, 1033, 948, 880, 691 cm−1; for 13C and 1H NMR spectroscopic data, see Tables 1 and 2; Negative FABMS m/z (rel. int.): 501 [M+H]+ (15); 339 (100), 325 (60); Negative HRESIMS m/z 501.3227 [M+H]+ (24) (calcd. for C30H47O6, 501.3216).

4.3.5. 16-oxolycoclamitin-29-yl E-4′-hydroxy-3′-methoxycinnamate (5)

White powder, m.p. 294–296 °C; [α]D23 +3.22 (MeOH; c 0.77); IR (KBr) νmax 3440 (OH), 2967, 1710, 1652, 1631 1596, 1513, 1387, 1231, 1190, 1033, 948, 880, 849, 817, 691 cm−1; for 13C and 1H NMR spectroscopic data, see Tables 1 and 2; Negative FABMS m/z (rel. int.): 679 [M+H]+ (100), 589 (25), 339 (100), 193 (70); Negative HRFABMS m/z 679.3843 [M+H]+ (calcd. for C40H53O18, 679.3846).

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References


Haruo, S., Kazuo, F., Xu, G.Y., Cao, X., Pan, D.J., 1988. Assignment of the1 H-and13 C NMR spectra of four lycopodium triterpenoids by the


