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Five new subspicatins and noreremophilane from *Parasenecio petasitoides* collected in China

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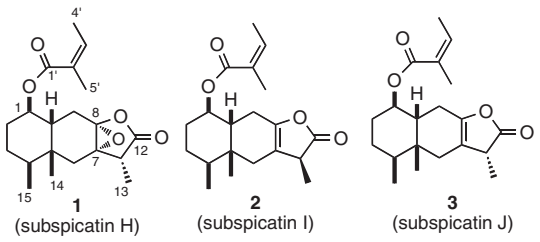
ABSTRACT

Five new 1β-angeloyloxyeremophilanolides (subspicatins H–L) and 1β-angeloyloxytetranoreremophilanone (norsubspicatin A), were isolated from the EtOAc extracts of *Parasenecio petasitoides* and their structures established on the basis of spectroscopic analyses. They are biogenetically mutually related to each other and represent a new category in the *Parasenecio* group. These findings suggest that there is diversity in this species, too.

We have been investigating both the inter- and intra-specific diversity of *Ligularia* species collected in the area of Hengduan Mountains in China and have reported our results.[1–7] This area is rich in plant species and very interesting from the viewpoint of plant diversity. We had the opportunity to collect *Parasenecio petasitoides* (Asteraceae), which used to be included in the genus *Cacalia*,[8] in 2008 in China. There are many reports of studies on these plants including one paper investigating the chemical constituents of the whole part of the same species.[9] They isolated germacrane- and guaiane-type sesquiterpenoids, but no eremophilane.[9] There is a nice review about the genus *Cacalia*, from which more than 100 terpenoids, alkaloids, and other aromatic compounds have been isolated.[10]

We found six new eremophilanes substituted with an angelate ester at C-1 position, all of which were biogenetically closely related. Eremophilane-type sesquiterpenoids bearing an angelate at C-1 position, named subspicatins A–G,[5,7] were previously found in *Ligularia subspicata* and *Ligularia lamarum*. The new compounds **1**–**6**, possessing the eremophilane- and noreremophilane-type skeleton, were very close to these subspicatins. The known compounds subspicatin C,[5] fukinone,[11] nerolidol, eremophil-11-en-8-one,[12] tussilagone,[13] and *O*-geranylconiferyl alcohol,[14] were also isolated. Because these compounds, found in both *Ligularia* and *Parasenecio* species, are biogenetically closely related, it is very interesting to discuss the relationships between *Ligularia* and *Parasenecio*.[15] We herein report the results of these studies in detail.[16]



Subspicatin H (**1**)[17] showed a quasi-molecular ion peak at *m/z* 349 and its molecular formula was determined to be $C_{20}H_{28}O_5$ by HRCIMS spectrum. The ^{13}C NMR and HSQC spectra indicated the presence of five methyl, four methylene, five methine, and six quaternary carbon signals. The IR spectrum indicated the presence

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of an epoxy or an enol lactone (1805 cm^{-1}).[5–7] The ^1H NMR spectrum (Table 1) showed the presence of angeloyl moiety (δ 1.82, 1.98, and 5.69), which was supported by the absorption at 1713 cm^{-1} in its IR spectrum. Because the presence of two carbonyl groups (δ 166.8, 175.5) and two olefinic carbons (δ 128.4, 137.8) was shown and the degree of unsaturation was seven, this molecule should be tetracyclic. 2D NMR analysis clearly indicated the eremophilane skeleton and the angelate group attached to C-1 position (Fig. 1). The ring C should be a lactone with an epoxide at C-7 and 8 positions, because the chemical shift for C-8 appeared at δ 86.3, a similar position to those previously isolated.[5–7] Furthermore, HMBC correlations from the C-13 methyl group to carbons at C-7, C-11, and C-12 were observed. These observations indicated that the ring C should be the epoxy lactone as depicted in the figure. The stereochemistry was determined by the NOEs between H-1 and H-6α, H-10 and H-14, H-10 and H-15, H-14 and H-6β, and H-6β and H-11, indicating that rings A and B were cis fused, and that H-13 and the epoxide ring were both in α-orientation.[18] The cis β-arrangement of both H-14 and H-15 was also determined. This type of compound has previously been isolated from *Ligularia*,[5–7] *Farfugium* and so on,[18,19] and is presumably derived from the corresponding furanoeremophilane by oxidation (vide infra).

Subspicatin I (**2**)[20] had a molecular formula of $C_{20}H_{28}O_4$ determined by HRCIMS spectrum. The ^{13}C NMR and HSQC spectra indicated the presence of five methyl, four methylene, five methine, and six quaternary carbon signals. Since there are two carbonyl (δ 167.2, 179.2) and four olefinic (δ 113.2, 128.3, 138.1, 146.1) carbons and the degree of unsaturation is seven, this compound should be tricyclic. The IR spectrum showed absorption at 1798 cm^{-1}, indicating the presence of an enol lactone, supported by the above-mentioned ^{13}C NMR data. The angelate moiety was shown by the signals of δ_H 1.85, 1.96, 5.70 and δ_C 128.3, 138.1 in its NMR spectra (Table 1), as well as the IR absorption at 1713 cm^{-1}. The position of the angelate ester at C-1 was indicated by the 2D NMR spectra. The ring C was an enol lactone as depicted in the figure, which was also supported by the 2D NMR analysis. The stereochemistry of the methyl group at C-11 was determined to be β-oriented, because the NOE between H-11 and H-6α was observed. The H-6β was correlated with the methyl group at C-5, which also showed the NOE with H-10, indicating the cis arrangement for rings A and B. The cis β-arrangement of both H-14 and H-15 was also determined by the NOE between H-10 and H-15. The angelate group at C-1 should be β-oriented, because the NOE between H-6α and H-1α was observed.

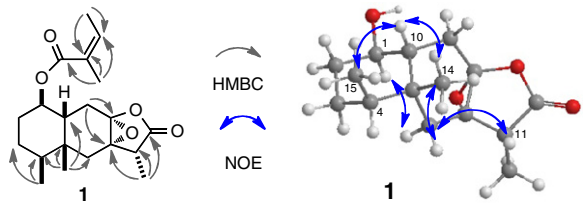

Figure 1. The major HMBC and NOESY correlations detected for subspicatin H (**1**) (angeloyloxy-group (Ang) was replaced with OH for clear display).

Subspicatin J (**3**)[21] showed very similar spectral data to those of subspicatin I (**2**). This compound also was an enol lactone (1796 cm^{-1}), with angelate moiety at the C-1 position. By comparing 2D spectral data of compounds **2** and **3**, these two compounds were revealed to be isomers due to the configuration of the C-13 methyl group. NOEs between H-10 and H-14, H-10 and H-15, H-6α and H-1α, and H-6β and H-14 were observed, indicating that rings A and B were cis-fused. The NOE between H-11α and H-6α was observed in the case of compound **2**, while the NOE between H-13 and H-6α was observed in the case of compound **3**. From these observations the structure of compound **2** was established to have 11αH and that of compound **3** to have 11βH, respectively (Fig. 2).

Subspicatins K (**4**)[22] and L (**5**)[23] showed similar spectroscopic data. The molecular formula for compound **4** was $C_{20}H_{28}O_4$, while that for **5** was $C_{20}H_{28}O_5$, as determined by the HRCIMS spectra. The presence of angelate ester at the C-1 position and the eremophilanolide structure (1759 cm^{-1}) were suggested by the HMBC spectra for both compounds. The difference between these two compounds was the presence of a hydroxy group (3379 cm^{-1}) and a quaternary carbon signal at δ 103.0 (C-8) for compound **5**. The eremophilane skeleton was suggested for both compounds by the HMBC spectra (Figs. 3 and 4). Therefore, compound **4** is 1-angeloyloxyeremophilenolide and compound **5** is 1-angeloyloxy-8-hydroxyeremophilenolide. The stereochemistry was determined by the NOESY spectra. The cis-fused A/B rings were determined by the NOE between H-10 and H-14 for both compounds. The coupling pattern of H-1 of compound **4** is a triplet of doublets (J = 11.2 and 4.6 Hz), indicating that H-1α is axial and rings A and B adopt non-steroidal conformation (Fig. 3). The NOE between H-1α and H-8α clearly indicated the hydrogen on C-8 was α for compound **4**. The NOE between H-15 and H-10 along with the results

Table 1
^1H NMR data of compounds **1**–**6** (500 MHz, measured in C_6D_6) (multi, J (Hz))

Position	1	2	3	4	5	6
1	5.13 (td, 11.4, 4.9)	4.80 (td, 11.0, 4.4)	4.80 (td, 10.7, 4.6)	4.96 (td, 11.2, 4.6)	4.83 (br s)	4.94 (q, 2.7)
2	1.99–2.04 (m)	1.95–2.02 (m)	1.83–1.89 (m)	1.73 (ddt, 11.8, 4.6, 3.5)	1.45 (br d, 13.9)	1.48–1.54 (m)
	1.16–1.24 (m)	1.35–1.43 (m)	1.34–1.43 (m)	1.32–1.41 (m)	1.20 (tt, 13.9, 4.0)	1.16 (tt, 13.9, 3.5)
3	1.37 (tt, 14.2, 4.2)	1.65–1.72 (m)	1.54–1.60 (m)	1.47 (tt, 13.9, 3.5)	1.32 (qd, 13.9, 3.6)	1.20–1.29 (m)
	0.97 (dtd, 14.2, 3.6, 2.4)	1.13 (br d, 14.7)	1.05–1.10 (m)	1.04–1.08 (m)	0.91–0.98 (m)	0.84–0.91 (m)
4	0.83–0.89 (m)	1.03–1.12 (m)	1.07–1.11 (m)	0.90–0.96 (m)	0.82–0.88 (m)	0.75–0.83 (m)
6	1.74 (d, 15.4)	1.89 (d, 14.9)	2.01–2.07 (m)	1.84 (br d, 15.2)	2.24 (d, 13.9)	2.09 (d, 17.9)
	0.55 (d, 15.4)	0.94 (d, 14.9)	0.89 (d, 16.1)	1.40 (d, 15.2)	1.91 (dq, 13.9, 1.4)	1.48 (d, 17.9)
8	–	–	–	4.79 (br dd, 10.8, 6.8)	–	–
9	2.56 (d, 15.4)	2.38 (d, 17.8)	2.35 (d, 17.9)	2.29 (ddd, 13.2, 6.8, 2.7)	2.14 (dd, 13.4, 3.9)	1.93 (dd, 17.9, 8.5)
	1.81 (dd, 15.4, 6.6)	1.95–2.02 (m)	1.96–2.02 (m)	1.06–1.12 (m)	1.40 (t, 13.4)	1.69 (dd, 17.9, 12.0)
10	1.51 (dd, 11.4, 6.6)	1.66 (dd, 11.0, 5.6)	1.64–1.68 (m)	1.42–1.48 (m)	2.19 (br d, 13.4)	1.80–1.89 (m)
11	2.02 (q, 7.3)	2.44–2.50 (m)	2.45–2.50 (m)	–	–	–
13	1.14 (3H, d, 7.3)	0.91 (3H, d, 7.6)	1.00 (3H, d, 7.6)	1.60 (3H, t, 1.4)	1.63 (3H, d, 1.4)	–
14	0.36 (3H, s)	0.56 (3H, s)	0.61 (3H, s)	0.28 (3H, s)	0.94 (3H, s)	0.78 (s)
15	0.63 (3H, d, 7.1)	0.74 (3H, d, 7.1)	0.73 (3H, d, 7.1)	0.64 (3H, d, 7.3)	0.49 (3H, d, 6.6)	0.48 (d, 6.6)
3′	5.69 (qq, 7.4, 1.5)	5.70 (qq, 7.2, 1.5)	5.68 (qq, 7.4, 1.5)	5.73 (qq, 7.2, 1.5)	5.75 (qq, 7.1, 1.5)	5.71 (qq, 7.1, 1.4)
4′	1.98 (3H, dq, 7.4, 1.5)	1.96 (3H, dq, 7.2, 1.5)	1.95 (3H, dq, 7.4, 1.5)	1.99 (3H, dq, 7.2, 1.5)	2.02 (3H, dq, 7.1, 1.5)	2.01 (dq, 7.1, 1.4)
5′	1.82 (3H, quint, 1.5)	1.85 (3H, quint, 1.5)	1.83 (3H, quint, 1.5)	1.85 (3H, quint, 1.5)	1.83 (3H, quint, 1.5)	1.82 (quint, 1.4)

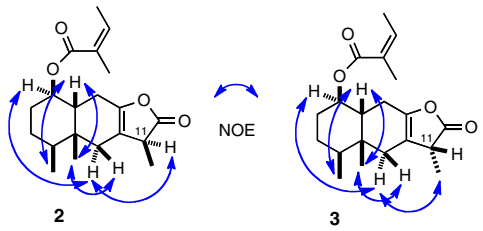

Figure 2. The major NOESY correlations detected for subspicatins I (**2**) and J (**3**).

mentioned above indicated that both H-14 and H-15 are β-orientated. Unfortunately, the coupling pattern of the H-1 for compound **5** was not clear. However, the NOEs between H-4α and H-9α, H-13 and H-6α, H-14 and H-10, H-14 and H-6β, H-6β and H-10, and H-9β and H-1α were observed, indicating that this compound adopted steroidal conformation with A/B cis-fused rings and C-8β OH configuration as well as cis β-arrangement of both H-14 and H-15.

Compound **6**[24] had the molecular formula $C_{16}H_{24}O_3$, suggesting that it had four carbons less than the other compounds. The IR spectrum showed two carbonyl absorptions at 1742 and 1713 cm^{-1}, supported by the ^{13}C NMR signals at δ 213.9 and 166.5. The tetranoreremophilane skeleton was suggested by the 2D NMR spectra. The carbonyl group at C-8[25] and the angelate moiety at C-1 positions were also determined by the HMBC spectrum (Fig. 5). The stereochemistry was established by the NOEs between H-14 and H-10, H-1α and H-9β, H-15 and H-6α, H-15 and H-3β, and H-14 and H-3β (Fig. 5). The structure of compound **6** was established as depicted in the formula and was named norsubsticatin A.

Cacalia (=*Parasenecio*) *decomposita*[26] is known to produce cacalol as the main constituent. Other *Cacalia* species do not necessarily produce cacalol itself, but its derivatives.[27] *Parasenecio deltophylla* produces highly-oxidized cacalol derivatives.[8] In contrast, cacalol or its derivatives were not obtained from *P. petasitoides* at all, but subspicatin C[5] as a major constituent as well as other new subspicatins were isolated. Norsubspicatin A (**6**) may be derived from subspicatin C through the plausible route shown in Figure 6. An alternative route from a non-furano type compound, such as **7**, followed by oxidation to C-1 *O*-angelate, could not be eliminated, although no C-1 oxidized non-furano type of compound was isolated in our studies.

Subspicatins (1-angeloyloxyfuranoeremophilane and its derivatives) were previously isolated as characteristic compounds of *L. subspicata* and *L. lamarum*.[5,7] The compounds were not obtained from the other *Ligularia* species collected in the Hengduan Mountains to date. Thus, *P. petasitoides* was very close to *L. subspicata* and *L. lamarum* in chemical composition. In the course of our research project, diversity in chemical composition was found in a number of *Ligularia* species, and was thought to be generated by hybridization or introgression.[28] Since *Ligularia* and *Parasenecio*

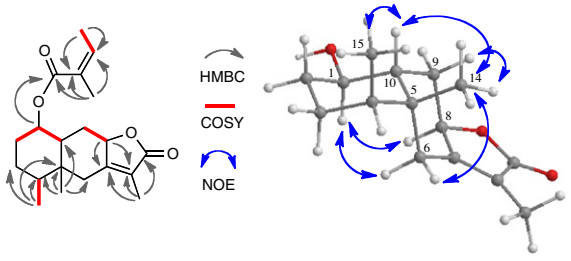

Figure 3. Selected HMBC and NOESY correlations for compound **4** (Ang was replaced with OH for clear display).

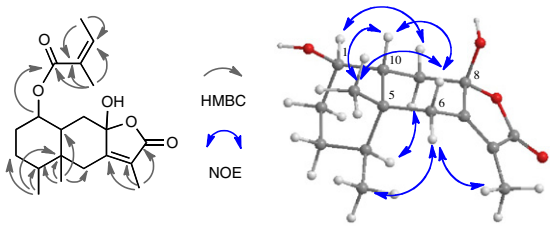

Figure 4. Selected HMBC and NOESY correlations for compound **5** (Ang was replaced with OH for clear display).

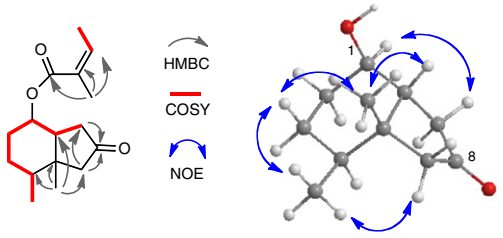

Figure 5. Selected HMBC and NOESY correlations for compound **6** (Ang was replaced with OH for clear display).

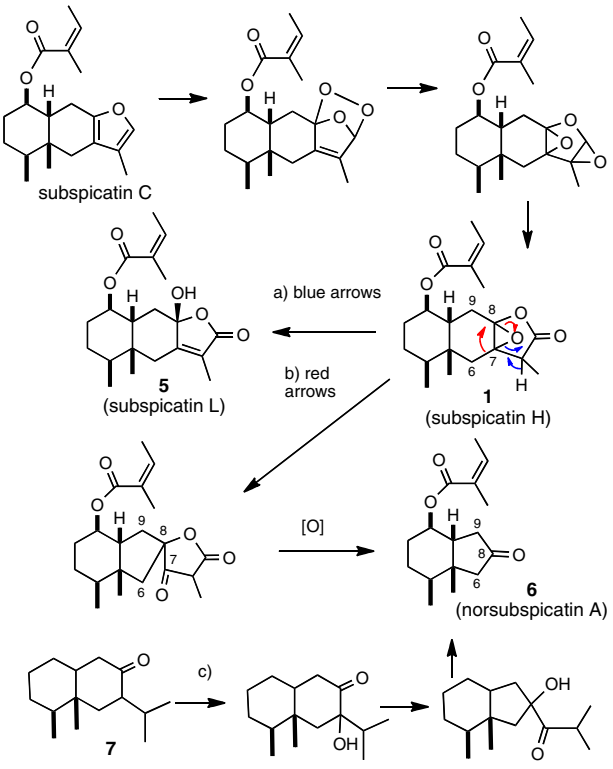

Figure 6. Plausible biogenetic pathways.

are genetically close to each other,[15] the production of subspicatins in *P. petasitoides* is likely to be the result of genetic correlation with *L. subspicata* or *L. lamarum*. Indeed, subspicatins were isolated from *L. lamarum* samples collected in the same County.[7] It will thus be very interesting to investigate more *Parasenecio* species in order to understand their chemical diversity. We plan to collect more samples in the Hengduan Mountains area in the near future for further studies.

Acknowledgments

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16. The root of *Parasenecio petasitoides*, collected in Madeng, Yunnan (N: 26°31′9″, E: 99°42′25″, Alt: 2686 m) in 2008 (voucher specimen, No. 200861, deposited in the Herbarium of Kunming Institute of Botany) was identified by X. Gong, one of the authors, and extracted with EtOAc to give an extract (423 mg), which was separated by a silica-gel column chromatography (hexane/AcOEt, in gradient) followed by HPLC (Nucleosil 50-5, hexane/AcOEt) to isolate **1** (1.2 mg), **2** (0.9 mg), **3** (0.7 mg), **4** (3.8 mg), **5** (3.9 mg), and **6** (0.9 mg), as well as subspicatin C (92.3 mg), fukinone (0.5 mg), nerolidol (6.8 mg), eremophil-11-en-8-one (0.5 mg), tussilagone (0.9 mg), *O*-geranylconiferyl alcohol (1.3 mg).
17. Subspicatin H (**1**): $[\alpha]_D$ −42.9 (*c* 0.12, EtOH). CD [θ] (nm) (EtOH): −9760 (225), −3580 (292). IR (KBr): 1805, 1713 cm^{-1}. ^{13}C NMR (125 MHz, C_6D_6) δ 10.5 (C-13), 14.3 (C-15), 15.9 (C-4′), 19.7 (C-9), 20.9 (C-5′), 25.6 (C-14), 26.7 (C-2), 26.7 (C-3), 32.7 (C-6), 36.5 (C-5), 36.8 (C-4), 38.5 (C-10), 44.5 (C-11), 63.2 (C-7), 71.7 (C-1), 86.3 (C-8), 128.4 (C-2′), 137.8 (C-3′), 166.8 (C-1′), 175.5 (C-12). MS (CI): *m/z* 349 [M+H$^+$], 331, 249, 231, 83 (100). HRMS(CI): *m/z* [M+H$^+$] calcd for $C_{20}H_{29}O_5$: 349.2015; found: 349.2022.
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20. Subspicatin I (**2**): $[\alpha]_D$ −45.6 (*c* 0.09, EtOH). IR (KBr): 1798, 1713 cm^{-1}. ^{13}C NMR (125 MHz, C_6D_6) δ 14.0 (C-13), 14.5 (C-15), 15.9 (C-4′), 20.6 (C-9), 20.9 (C-5′), 24.7 (C-14), 26.7 (C-2), 27.2 (C-3), 30.3 (C-6), 36.7 (C-5), 37.0 (C-4), 39.8 (C-10), 40.2 (C-11), 72.1 (C-1), 113.2 (C-7), 128.3 (C-2′), 138.1 (C-3′), 146.1 (C-8), 167.2 (C-1′), 179.2 (C-12). MS (CI): *m/z* 333 [M+H$^+$], 233 (100). HRMS(CI): *m/z* [M+H$^+$] calcd for $C_{20}H_{29}O_4$: 333.2065; found: 333.2065.
21. Subspicatin J (**3**): $[\alpha]_D$ −62.0 (*c* 0.06, EtOH). CD [θ] (nm) (EtOH): −3400 (212), +3690 (231). IR (KBr): 1796, 1713 cm^{-1}. ^{13}C NMR (125 MHz, C_6D_6) δ 14.4 (C-13), 14.5 (C-15), 15.9 (C-4′), 20.7 (C-9), 20.8 (C-5′), 24.7 (C-14), 26.8 (C-2), 27.1 (C-3), 30.5 (C-6), 36.8 (C-5), 36.8 (C-4), 40.0 (C-10), 41.4 (C-11), 71.7 (C-1), 113.2 (C-7), 128.4 (C-2′), 138.1 (C-3′), 146.1 (C-8), 167.1 (C-1′), 179.0 (C-12). MS (CI): *m/z* 332 [M]$^+$, 233 (100). HRMS(CI): *m/z* [M$^+$] calcd for $C_{20}H_{28}O_4$: 332.1988; found: 332.1982.
22. Subspicatin K (**4**): $[\alpha]_D$ −37.4 (*c* 0.38, EtOH). CD [θ] (nm) (EtOH): −13600 (225). IR (KBr): 1759, 1713 cm^{-1}. ^{13}C NMR (125 MHz, C_6D_6) δ 8.1 (C-13), 15.1 (C-15), 16.0 (C-4′), 20.8 (C-5′), 24.4 (C-14), 26.9 (C-2), 27.1 (C-3), 29.6 (C-9), 34.2 (C-6), 37.6 (C-4), 40.6 (C-5), 41.1 (C-10), 70.5 (C-1), 76.9 (C-8), 122.4 (C-11), 128.0 (C-2′), 138.6 (C-3′), 160.6 (C-7), 167.3 (C-1′), 173.7 (C-12). MS (CI): *m/z* 333 [M+H$^+$], 233 (100). HRMS(CI): *m/z* [M+H$^+$] calcd for $C_{20}H_{29}O_4$: 333.2066; found: 333.2063.
23. Subspicatin L (**5**): $[\alpha]_D$ +42.9 (*c* 0.39, EtOH). CD [θ] (nm) (EtOH): +17050 (209), −3630 (226), +19810 (243). IR (KBr): 3379, 1759, 1713 cm^{-1}. ^{13}C NMR (125 MHz, C_6D_6) δ 8.3 (C-13), 15.7 (C-15), 15.9 (C-4′), 20.9 (C-5′), 23.1 (C-14), 26.0 (C-2), 26.5 (C-3), 29.5 (C-4), 34.8 (C-6), 38.9 (C-9), 39.7 (C-5), 43.2 (C-10), 73.7 (C-1), 103.0 (C-8), 122.7 (C-11), 128.2 (C-2′), 138.7 (C-3′), 158.0 (C-7), 166.8 (C-1′), 172.4 (C-12). MS (CI): *m/z* 349 [M+H$^+$], 248, 231 (100). HRMS(CI): *m/z* [M+H$^+$] calcd for $C_{20}H_{29}O_5$: 349.2015; found: 349.2006.
24. Norsubspicatin A (**6**): $[\alpha]_D$ −60.8 (*c* 0.05, EtOH). CD [θ] (nm) (EtOH): −4590 (299). IR (KBr): 1742, 1713 cm^{-1}. ^{13}C NMR (125 MHz, C_6D_6) δ 15.9 (C-4′), 16.0 (C-15), 20.9 (C-14), 21.0 (C-5′), 25.2 (C-3), 25.6 (C-2), 33.4 (C-4), 40.1 (C-9), 40.3 (C-5), 46.2 (C-10), 52.8 (C-6), 70.4 (C-1), 128.5 (C-2′), 138.4 (C-3′), 166.5 (C-1′), 213.9 (C-8). MS (CI): *m/z* 265 [M+H$^+$], 193, 165 (100). HRMS(CI): *m/z* [M+H$^+$] calcd for $C_{16}H_{25}O_3$: 265.1803; found: 265.1815.
25. The numbering system follows biosynthetic considerations (Fig. 6). The methyl group at C-5 (H-14) is axial for ring A, and that at C-4 (H-15) equatorial in compound **6** (both are β-orientation), whose conformation is very similar to that of compound **5**.
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