Study on Chemical Constituents from *Salacia amplifolia*

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ABSTRACT: OBJECTIVE To investigate the chemical constituents of *Salacia amplifolia*. **METHODS** The chemical constituents were isolated and purified by silica gel and Sephadex LH-20 column chromatography, their structures were elucidated by chemical and spectral methods. **RESULTS** Nine compounds were isolated and their structures was determined as 2-hydroxyfriedelan-3-one (**1**), friedelin (**2**), lup-20 (29)-en-3, 21-dione (**3**), D-friedoolean-14-en-3-one (**4**), 3-(3″, 4″-dihydroxy-trans-cinnamoyloxy)-D-friedoolean-14-en-28-oic acid (**5**), 3, 22-dioxo-29-normoretane (**6**), Lupeol (**7**), β-Sitosterol (**8**), β-Daucosterol (**9**). **CONCLUSION** Compounds 1-9 are isolated for the first time from the plant.
KEY WORDS: *Salacia amplifolia*; chemical constituents; triterpenoids

阔叶五层龙的化学成分研究

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摘要：目的 对阔叶五层龙所含化学成分进行研究。**方法** 应用液-液萃取及多种柱层析方法进行化学成分的分离、纯化，并利用多种光谱技术进行结构的分析鉴定。**结果** 从阔叶五层龙植物中分离得到 9 个化合物：2-hydroxyfriedelan-3-one (**1**)，软木三萜酮 (**2**), lup-20(29)-en-3, 21-dione (**3**)，D-friedoolean-14-en-3-one (**4**)，3-(3″,4″-dihydroxy-trans-cinnamoyloxy)-D-friedoolean-14-en-28-oic acid (**5**), 3, 22-dioxo-29-normoretane (**6**), 羽扇豆醇 (**7**), β-谷甾醇 (**8**), β-胡萝卜苷(**9**)。**结论** 该 9 个化合物均为首次从阔叶五层龙中分离得到。
关键词：阔叶五层龙；化学成分；三萜类化合物
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The genus *Salacia*, belonging to the family Hippocrateaceae, is composed of more than 100 species distributed in the tropics, of which 10 species grow in Hainan, Guangdong, and Guangxi provinces[1]. The extractions of their stem or root have significant anti-oxidation, liver protection, lipid-lowering diet, and hypoglycemic activities. The main active ingredient are found to be pentacyclic triterpenoids including the derivatives of friedelane, lupinane, quinone methide[2]. As a specific plant in China, there is no report about the systematic chemical study of *Salacia amplifolia* Merr. ex Chun & F. C.. How we

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study on the chemical composition of *Salacia amplifolia* to demonstrate the efficacy of the material basis and to improve the research and development of the plant. Here we report the isolation and identification of 9 compounds: 2-hydroxyfriedelan-3-one (**1**), friedelin (**2**), lup-20(29)-en-3, 21-dione (**3**), D-friedoolean-14-en-3-one (**4**), 3-(3″, 4″-dihydroxy-trans-cinnamoyloxy)-D-friedoolean-14-en-28-oic acid (**5**), 3, 22-dioxo-29-normoretane (**6**), Lupeol (**7**), β-Sitosterol (**8**), β-Daucosterol (**9**). All above were isolated from the plant for the first time.

1 General experimental procedures

XT-4 Microscope melting point apparatus (the temperatures were not corrected); Nicolet Impact 410 Infrared Spectrophotometer (KBr); Brucker ACF300 and AV500 NMR spectrometer; HP-1100 LC/MSD System (ESI Mode); YMC GEL ODS-A (50 μm), Sephadex LH-20 (Pharmacia), Column chromatography and TLC(Qingdao Chemical Factory), Reagents are analytically reagent.

2 Plant material

The plant of *S. amplifolia* was collected from the Gan Shi Ling nature reserve of Hainan province. The plant was authenticated by HUANG Shi Man, College of Life Science and Agriculture, Hainan University.

3 Extraction and Separation

Dried and crushed plant materials of *Salacia amplifolia* (10 kg) were refluxing extracted in 95% ethanol (2 h) for three times. The extract was filtered and concentrated. The concentrated extract was dissolved in water and extracting with petroleum ether followed by ethyl acetate and n-butanol to get petroleum ether extract (150 g), ethyl acetate extract (88 g). The latter was separated on silica gel eluted gradiently with petroleum ether-acetone and chloroform-methanol, and then purified with Sephadex LH-20 chromatography, the following compounds were obtained after recrystallization: **1**(50 mg), **2**(23 mg), **3**(40 mg), **4**(26 mg), **5**(17 mg), **6**(80 mg), **7**(130 mg), **8**(1 g), **9**(300 mg).

3.1 Compound **1**

Colorless crystals (chloroform), mp 247−249 ℃. Liebermann-Burchard positive reaction. ESI-MS *m/z* 441[M-H]$^-$. According to ^{1}H-NMR and ^{13}C-NMR the molecular formula was $C_{30}H_{50}O_2$. ^{1}H-NMR ($CDCl_3$, 500MHz) δ 0.92 (3H, s), 0.95 (3H, s), 1.03 (3H, s), 1.05 (3H, s), 1.08 (3H, s), 1.12(3H, s), 2.27 (2H, m, H-1), 2.40 (1H, m, H-4), 3.72 (1H, dd, *J*=4.35 Hz, H-2); ^{13}C-NMR ($CDCl_3$, 500 MHz) δ 213.18 (C-3), 74.28 (C-2), 59.45 (C-10), 58.16 (C-4), 51.49 (C-8), 46.95 (C-18), 44.27 (C-6), 42.04 (C-5), 41.45 (C-22), 41.20 (C-16), 38.97 (C-13), 38.78 (C-14), 37.45 (C-9), 36.06 (C-11), 35.92 (C-19), 35.24 (C-30), 34.33 (C-17), 33.10 (C-1), 32.46 (C-20), 31.85 (C-21), 30.38 (C-15), 30.14 (C-28), 24.88(C-29), 22.24 (C-12), 19.25 (C-26), 18.21 (C-27), 18.18 (C-7), 17.69 (C-25), 14.63 (C-24), 6.79 (C-23). The compound was identified as 2-hydroxyfriedelan-3-one because all the data above were coincident with the literature [3].

3.2 Compound **2**

Colorless crystals (chloroform), mp 242-245 ℃. Liebermann-Burchard positive reaction. ESI-MS *m/z* 426 [M$^+$]. According to ^{1}H-NMR and ^{13}C-NMR the molecular formula was $C_{30}H_{50}O$. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.75 (3H, s), 0.89 (3H, s), 0.91 (3H, s), 0.95 (3H, s), 0.98 (3H, s), 1.02 (3H, s), 1.03 (3H, s), 1.20 (3H, s), 1.98 (2H, m, H-1), 2.32 (2H, m, H-2), 2.40 (1H, m, H-4); ^{13}C-NMR ($CDCl_3$, 500 MHz) δ 213.62 (C-3), 59.49 (C-10), 58.23 (C-4), 53.11 (C-8), 42.80 (C-18), 42.14 (C-5), 41.53 (C-2), 41.30 (C-6), 39.70 (C-13), 39.25 (C-22), 38.30 (C-14), 37.45 (C-9), 36.01 (C-11), 35.62 (C-19), 35.35 (C-29), 35.04 (C-15), 32.78 (C-21), 32.42 (C-16), 32.08 (C-28), 31.7 (C-30), 30.50 (C-12), 30.00(C-17), 28.17 (C-20), 22.27 (C-1), 20.25 (C-26), 18.65 (C-27), 18.23 (C-7), 17.94 (C-5), 14.65 (C-24), 6.81 (C-23). The compound was identified as friedelin because all the data above were coincident with the literature [4].

3.3 Compound **3**

Colorless crystals (chloroform), mp 170-172 ℃. Liebermann-Burchard positive reaction. ESI-MS *m/z* 437 [M-H]$^-$. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.92 (3H, s), 0.95 (3H, s), 1.03 (3H, s), 1.05 (3H, s), 1.08 (3H, s), 1.12 (3H, s), 4.81 (1H, s, H-30), 4.93 (1H, s,H-30′), 3.82 (1H, d, *J*=14.8 Hz, H-19), 1.65 (3H, s, H-29); ^{13}C-NMR ($CDCl_3$, 500 MHz) δ 217.79 (C-21), 217.65 (C-3), 143.46 (C-20), 114.96 (C-30), 59.03 (C-19), 55.40 (C-5), 54.95 (C-22), 49.61 (C-9), 47.31 (C-4), 47.00 (C-18), 42.75 (C-14), 40.89 (C-8), 39.58 (C-1), 37.85 (C-17), 37.31 (C-13), 36.88 (C-10), 34.84 (C-2), 34.08 (C-16), 33.28 (C-7), 26.93 (C-15), 26.63 (C-12), 25.36 (C-24), 21.26 (C-28), 21.02 (C-23), 20.84 (C-29), 19.59 (C-11), 18.17 (C-6), 15.91 (C-26), 15.76 (C-25), 14.51 (C-27). The compound was identified as lup-20(29)-en-3,21-dione

(salacianone) because all the data above were coincident with the literature [5].

3.4 Compound **4**

Colorless crystals (chloroform), mp 240-243 ℃. Liebermann-Burchard positive reaction. ESI-MS *m/z*: 447.3 $[M+Na]^+$. The molecular formula $C_{30}H_{50}O$ which was supported by NMR. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.56(dd, *J*=8.1, 3.3 Hz, H-15), 2.57 (m, H-2), 2.31 (m, H-2′), 1.14 (3H, s), 1.09 (3H, s), 1.07 (3H, s), 1.06 (3H, s), 0.97(3H, s), 0.92 (6H, s, 2×CH_3), 0.83 (3H, s); ^{13}C-NMR ($CDCl_3$, 300 MHz) δ: 38.8 (C-1), 33.9 (C-2), 217.5 (C-3), 47.8 (C-4), 55.9 (C-5), 21.3 (C-6), 35.6 (C-7), 39.2 (C-8), 49.1 (C-9), 35. 1 (C-10), 17.5 (C-11), 38.9 (C-12), 38.1 (C-13), 157.9 (C-14), 117.4 (C-15), 37.1 (C-16), 37.7 (C-17), 48.9 (C-18), 40.9 (C-19), 28.9 (C-20), 33.9 (C-21), 33.4 (C-22), 26.1 (C-23), 21.6 (C-24), 15.1 (C-25), 29.9 (C-26), 26.0(C-27), 30.3 (C-28), 33.1 (C-29), 20.8 (C-30). The compound was identified as D- friedoolean-14-en-3-one (Taraxerone) because all the data above were coincident with the literature [6].

3.5 Compound **5**

Light yellow amorphous (chloroform-methanol), mp 196-198 ℃, Vanillin concentrated oil of vitriol reaction significant purple, $FeCl_3$ significant purple blue, UV 365 nm blue-fluorescence. Liebermann-Burchard positive reaction, tips for Triterpenes. ESI-MS *m/z* 617$[M-H]^-$. According to ^{1}H-NMR and ^{13}C-NMR the molecular formula was $C_{39}H_{53}O_6$. ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 7.53 (1H, d, *J*=16 Hz, H-3′), 6.28 (1H, d, *J*=16 Hz, H-2′), 7.04 (1H, d, *J*=2 Hz, H-2″), 7.01 (1H, dd, *J*=8.2 Hz, 2Hz, H-6″), 6.77 (1H, d, *J*=8.2 Hz, H-5″), 5.58 (1H, dd, *J*=8 Hz, 3.5 Hz, H-15), 4.67(1H, t, *J*=2.5 Hz, H-3); ^{13}C-NMR ($CDCl_3$, 500 MHz), δ 183.3 (C-28), 167.2 (C-1′), 161.0 (C-14), 151.1 (C-4″), 146.0 (C-3′), 145.4 (C-3″), 127.9 (C-1″), 122.3 (C-6″), 117.8(C-5″), 116.7 (C-15), 114.8(C-2″), 78.5 (C-3), 51.4 (C-17), 50.7 (C-5), 50.3 (C-9), 41.6 (C-18), 40.9 (C-7), 39.2 (C-8), 38.1 (C-4), 37.4 (C-13), 36.8 (C-10), 36.7 (C-1), 34.3 (C-19), 33.7 (C-21), 33.5 (C-12), 32.2 (C-16), 32.0 (C-29), 31.9 (C-22), 30.4 (C-20), 29.3 (C-30), 27.9 (C-23), 26.2 (C-26), 22.8 (C-2), 22.5 (C-27), 21.8 (C-24), 18.6 (C-6), 17.3 (C-11), 15.4 (C-25). The compound was identified as 3-(3″,4″-Dihydroxy-trans-cinnamoyloxy)-D-friedoolean-14-en-28-oic acid because all the data above were coincident with the literature [7].

3.6 Compound 6

Colorless crystals (chloroform), mp 260-263 ℃. Liebermann-Burchard positive reaction. ESI-MS *m/z* 426 $[M]^+$. According to ^{1}H-NMR and ^{13}C-NMR the molecular formula was $C_{29}H_{46}O_2$. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.78 (3H, s), 0.92 (3H, s), 0.97 (3H, s), 1.02 (3H, s), 1.05 (3H, s), 1.07 (3H, s), 2.15 (3H, s, $COCH_3$-30), 2.44 (2H, m, $COCH_2$-2), 2.59 (1H, dt, H-21); ^{13}C-NMR ($CDCl_3$, 500 MHz) δ 218.1 (C-3), 212.7 (C-22), 54.7(C-21), 52.5(C-5), 49.5(C-9), 49.4 (C-13), 47.2(C-4), 43.0(C-14), 42.7(C-8), 40.6(C-14), 39.7(C-13), 39.4(C-2), 37.0(C-18), 36.8(C-10), 34.8 (C-1), 34.0(C-17), 33.4(C-7), 29.2(C-19), 27.6(C-15), 27.2(C-29), 27.1(C-20), 26.7(C-19), 21.4(C-24), 21.0 (C-6), 19.6(C-26), 17.9(C-23), 15.9(C-27), 15.6(C-25), 14.3(C-28). The compound was identified as 3, 22-dioxo-29-normoretane because all the data above were coincident with the literature [8].

3.7 Compound **7**

Colorless crystals (chloroform), mp 204~206 ℃. Liebermann-Burchard positive reaction. ESI-MS *m/z* 425$[M-H]^-$. According to ^{1}H-NMR and ^{13}C-NMR the molecular formula was $C_{30}H_{50}O$. ^{1}H-NMR($CDCl_3$, 300 MHz) δ 0.82(3H, s), 0.83(3H, s), 0.86(3H, s), 0.99(3H, s), 1.13(3H, s), 1.16(3H, s), 1.65(3H, s), 3.23(1H, dd, *J*=10.5, 5.7 Hz, H-3), 4.59(1H, s, H-30′), 4.60(1H, s, H-30); ^{13}C-NMR($CDCl_3$,300MHz) δ 150.7 (C-20), 109.3(C-30), 78.9(C-3), 55.3(C-5), 50.4(C-9), 48.7(C-18), 46.8(C-19), 42.9(C-17), 42.6(C-14), 40.8(C-8), 40.2(C-22), 38.8(C-4), 38.3(C-1), 37.1(C-10), 36.9(C-13), 34.3(C-16), 34.2(C-7), 29.7(C-21), 27.4 (C-15), 27.35(C-2), 25.0(C-12), 21.6(C-23), 20.9 (C-11), 19.7(C-28), 19.5(C-29), 18.2(C-6), 16.0(C-25), 15.9(C-26), 15.3(C-24), 14.4(C-27). The compound was identified as Lupeol [9].

3.8 Compound **8**

Colorless crystals (chloroform), mp 140-142 ℃. Liebermann-Burchard positive reaction, Molish negative reaction. TLC verify with β-sitosterol, Rf and color consistent, melting test mixture, mp does not decline. The compound was identified as β-sitosterol.

3.9 Compound **9**

White crystals (chloroform-methanol), mp 281-283 ℃. Liebermann-Burchard positive reaction, Molish negative reaction. TLC verify with β-Daucosterol, Rf and color consistent, melting test mixture, mp does not decline. The compound was identified as β-Daucosterol.

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REFERENCES

[1] Institute of Botany of Guangdong Hainan Flora(海南植物志)[M].Vol 11. Beijing: Science Press, 1965: 443.

[2] YOSHIKAWA M, NINOMIYA K, SHIMODA H, et al. Hepatoprotective and antioxidative properties of Salacia reticulate: preventive effects of phenolic constituents on CCl_4^-induced liver inury in mice [J]. Biol Pharm Bull, 2002, 25(1): 72-76.

[3] MOITEIRO C, JUSTINO F, TAVARES R, et al. Synthetic secofriedelane and friedelane derivatives as inhibitors of human lymphocyte proliferation and growth of human cancer cell lines *in vitro* [J]. J Nat Prod, 2001, 64(10): 1273-1277.

[4] CHANG C W, WU T W, HSIEH Y S, et al. Terpenoids of *Syzygium formosanum* [J]. J Nat Prod, 1999, 62 (2): 327-328.

[5] A. HISHAM, G. JAYA KUMAR, Y.FUJIMOTO, et al. Salacianone and salacianol, two triterpenes from *salacia beddomei* [J]. Phytochemistry, 1995, 40(4): 1227-1231.

[6] VAN KIEM P, VAN MINH C, HUONG HT, et al. Pentacyclic triterpenoids from Mallotus apelta [J]. Arch Pharm Res, 2004, 27(11): 1109-1113.

[7] SULTANOVA N, MAKHMOOR T, YASIN A, et al. Isotamarixen–a new antioxidant and prolyl endopeptidase-inhibiting triterpenoid from tanarix hispida [J]. Planta Med, 2004, 70(1):65-67.

[8] HUI W H, LI M M. Triterpenoids from two Mallotus species: a nor-tritepene and two new acids [J]. Phytochemistry, 1976, 15(6): 985-986.

[9] WENKERT E, BADDELEY G V, BURFITT IR, et al. Carbon-13 nuclear magnetic resonance spectroscopy of naturally-occurring substances-LVII$^+$ Triterpenes Related to Lupane and Hopane [J]. Organic Magnetic Resonance, 1977, 11(7): 337-343.

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