Postprint: Chemical Constituents from the Twigs and Leaves of Croton lachnocarpus

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Abstract

To investigate the chemical constituents in the twigs and leaves of Croton laevigatus, this study employed a combination of chromatographic methods including silica gel, Sephadex LH-20 column chromatography, and HPLC to isolate compounds from the ethyl acetate fraction of the 95% ethanol extract of Croton laevigatus twigs and leaves. Eight compounds were obtained and identified as 2β-hydroxyteucvidin acetate (1), 2β-hydroxyteucvidin (2), crotoeurin B (3), kaempferol-3-O-(6 -O-cis-p-coumaroyl)-β-D-glucopyranoside (4), kaempferol-3-O-(6 -O-trans-p-coumaroyl)-β-D-glucopyranoside (5), castanoside A (6), cerevisterol (7), and uracil (8) through spectroscopic data analysis and literature comparison. Compounds 2–7 were isolated from this plant for the first time.

Full Text

Preamble

Chemical Constituents from the Twigs and Leaves of *Croton lachynocarpus*

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Abstract

To investigate the chemical constituents from the twigs and leaves of *Croton lachynocarpus*, this study employed various chromatographic techniques including silica gel column chromatography, Sephadex LH-20, and reversed-phase HPLC to separate the ethyl acetate fraction of a 95% ethanol extract. Eight compounds were isolated and identified through spectroscopic data analysis and comparison with literature reports as: 2β-hydroxyteucvidin acetate (1), 2β-hydroxyteucvidin (2), crotoeurin B (3), kaempferol-3-O-(6 -O-*cis*-p-coumaroyl)-β-D-glucopyranoside (4), kaempferol-3-O-(6 -O-*trans*-p-coumaroyl)-β-D-glucopyranoside (5), castanoside A (6), cerevisterol (7), and uracil (8). Compounds 2–7 were obtained from this plant for the first time.

Keywords: *Croton*; *Croton lachynocarpus*; chemical constituents

Introduction

Croton lachynocarpus, belonging to the Euphorbiaceae family, is a plant species known locally as "Xiaoye Shuanglonglong." It is primarily distributed in mountainous sparse forests or shrublands of Guangxi, Guangdong, southern Jiangxi, southern Hunan, and southern Guizhou. The young branches, leaves, inflorescences, and fruits are densely covered with stellate pubescence (Editorial Commission of China Flora of Chinese Academy of Sciences, 1996). Traditionally, the roots and leaves have been used in folk medicine for dispelling wind and dampness, dissipating blood stasis, relieving pain, reducing swelling, and detoxification, particularly for treating traumatic injuries and rheumatoid arthritis.

Our research group previously isolated triterpenoids, sterols, and phenolic acid esters from the roots of *C. lachynocarpus* (Pan et al., 2014; Pan et al., 2014), and identified various volatile oil components from the leaves that are potentially valuable for modern fragrance synthesis, including α-terpineol, eucalyptol, and α-bisabolene (Ning et al., 2013). However, research on the chemical constituents and pharmacology of this plant remains limited, restricting its application primarily to traditional Chinese medicine formulations and severely hindering its further development and utilization. To comprehensively elucidate its chemical basis and discover more bioactive compounds with development potential, this study further investigated the chemical constituents of the 95% ethanol extract from the twigs and leaves of *C. lachynocarpus*, leading to the isolation and identification of eight compounds. Among these, compounds 1–3 are diterpenoids, while compounds 4–6 are flavonoids. Their chemical structures are shown in Figure 1 [Figure 1: see original paper]. Compounds 2–7 were isolated from this plant for the first time.

1. Instruments and Materials

An AVANCE III HD 500 MHz superconducting NMR spectrometer (TMS internal standard, Bruker, Switzerland), LC/MS-IT-TOF mass spectrometer

(Shimadzu, Japan), and Agilent 1200 HPLC system were used. HPLC-grade reagents were purchased from Thermo Fisher Scientific (China) Co., Ltd., while analytical-grade reagents were from Xilong Chemical Co., Ltd. Silica gel for column and thin-layer chromatography was obtained from Qingdao Marine Chemical Co., Ltd., and Sephadex LH-20 gel was from Amershan Biosciences (Switzerland).

The twigs and leaves of *Croton lachynocarpus* were collected from Yangshuo County, Guilin City, Guangxi, and identified by Dr. Weibin Xu from the Guangxi Institute of Botany, Chinese Academy of Sciences. Voucher specimens are deposited at the Guangxi Key Laboratory of Functional Phytochemicals Research and Utilization.

2. Extraction and Separation

Air-dried and powdered twigs and leaves of *C. lachynocarpus* (8.0 kg) were extracted with 95% ethanol (40 L) at room temperature for 24 h. The mixture was filtered, and the residue was re-extracted twice using the same method. The combined extracts were concentrated under reduced pressure to yield 850 g of crude extract. An 800 g portion of the extract was suspended in water with ultrasonic dispersion and sequentially partitioned three times each with petroleum ether and ethyl acetate (2 L each). Concentration under reduced pressure afforded 144 g of petroleum ether fraction and 239 g of ethyl acetate fraction.

The ethyl acetate fraction (189 g) was subjected to silica gel column chromatography using a petroleum ether-acetone gradient (1:0\$→0 : 1)*aseluent.Theeluatesweremonitoredby TLCandcombinedintofourfractions*(*Fr*.1–*Fr*.4).*Fraction2wasfurth*
acetone = 8 : 1)*toyieldcompounds*1(9*mg*), 2(7.2*mg*), 7(15.4*mg*), *and*8(9.6*mg*).*Fraction3wasseparatedbysemi–*
preparativeHPLC(50_{3}$OH containing 0.1% HCOOH) to obtain compounds 5 (6.6 mg) and 6 (4.6 mg). Fraction 4 was purified by Sephadex LH-20 column chromatography using chloroform/methanol (1:1) to give compounds 3 (10.2 mg) and 4 (7.9 mg).

3. Structural Identification

Compound 1 was obtained as needle-like crystals. HR-ESI-MS m/z: 387.1816 $[M+H]^+$, molecular formula $C_{21}H_{22}O_7$. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 6.36 (1H, m, H-14), 5.37 (1H, t, J = 8.0 Hz, H-12), 2.55 (2H, dd, J = 8.5, 15.0 Hz, H-11b), 2.09 (3H, s, OAc), 1.94 (1H, dd, J = 8.0, 14.0 Hz, H-11a), 1.35 (3H, d, J = 7.0 Hz, H-17); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 28.0 (C-1, t), 66.8 (C-2, d), 24.9 (C-3, t), 124.1 (C-4, s), 161.7 (C-5, s), 76.0 (C-6, d), 35.9 (C-7, t), 38.9 (C-8, d), 51.9 (C-9, s), 33.8 (C-10, d), 39.0 (C-11, t), 72.0 (C-12, d), 125.1 (C-13, s), 108.0 (C-14, d), 144.6 (C-15, d), 139.6 (C-16, d), 14.3 (C-17, q), 171.4 (C-18, s), 176.9 (C-20, s), 169.9 (OC=O, s), 21.1 (COO-CH_3, q). These data are consistent with those reported in the literature (Savona et al., 1986). Therefore, compound 1 was identified as 2β-hydroxyteucvidin acetate.

Compound 2 was obtained as needle-like crystals. HR-ESI-MS m/z: 345.1788 $[M+H]^+$, molecular formula $C_{19}H_{20}O_6$. ^{1}H-NMR (500 MHz, C_5D_5N): δ 7.83 (1H, s, H-16), 7.70 (1H, s, H-15), 6.69 (1H, s, H-14), 5.52 (1H, t, J = 8.0 Hz, H-12), 2.66 (1H, dd, J = 8.0, 14.0 Hz, H-11b), 1.99 (1H, dd, J = 8.0, 14.0 Hz, H-11a), 1.39 (3H, d, J = 7.5 Hz, H-17); ^{13}C-NMR (125 MHz, C_5D_5N): δ 34.7 (C-1, t), 67.8 (C-2, d), 31.8 (C-3, t), 127.5 (C-4, s), 163.9 (C-5, s), 77.6 (C-6, d), 37.0 (C-7, t), 40.7 (C-8, d), 53.6 (C-9, s), 38.0 (C-10, d), 40.6 (C-11, t), 73.6 (C-12, d), 127.2 (C-13, s), 110.3 (C-14, d), 146.1 (C-15, d), 142.1 (C-16, d), 15.5 (C-17, q), 173.6 (C-18, s), 179.2 (C-20, s). These spectroscopic data are consistent with those reported in the literature (Savona et al., 1986). Therefore, compound 2 was identified as 2β-hydroxyteucvidin.

Compound 3 was obtained as needle-like crystals. HR-ESI-MS m/z: 361.1571 $[M+H]^+$, molecular formula $C_{20}H_{24}O_6$. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.46 (1H, br s, H-16), 7.42 (1H, br s, H-15), 6.41 (1H, br s, H-14), 3.10 (1H, dd, J = 15.0, 3.0 Hz, H-7b), 2.57 (1H, dd, J = 13.5, 6.5 Hz, H-11b), 2.34 (1H, dd, J = 13.5, 10.0 Hz, H-11a), 2.23 (1H, dd, J = 15.0, 5.5 Hz, H-7a), 2.16 (1H, m, H-4), 1.02 (1H, d, J = 6.5 Hz, H-17); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 23.1 (C-1, t), 25.5 (C-2, t), 24.9 (C-3, t), 42.3 (C-4, d), 47.4 (C-5, d), 208.8 (C-6, s), 45.4 (C-7, t), 32.9 (C-8, d), 51.5 (C-9, s), 42.6 (C-10, d), 36.5 (C-11, t), 70.7 (C-12, d), 124.0 (C-13, s), 108.2 (C-14, d), 144.3 (C-15, d), 140.0 (C-16, d), 17.0 (C-17, q), 173.7 (C-18, s), 177.0 (C-20, s), 51.4 (COO-CH_3, q). These spectroscopic data are consistent with those reported in the literature (Pan et al., 2015). Therefore, compound 3 was identified as crotoeurin B.

Compound 4 was obtained as a yellow powder. HR-ESI-MS m/z: 593.1383 $[M-H]^-$, molecular formula $C_{30}H_{26}O_{13}$. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.54 (2H, d, J = 8.0 Hz, H-2 , 6), 6.69 (2H, d, J = 8.0 Hz, H-3 , 5), 6.67 (1H, d, J = 13.0 Hz, H-7), 5.46 (1H, d, J = 13.0 Hz, H-8); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 156.5 (C-2, s), 133.0 (C-3, s), 177.3 (C-4, s), 161.2 (C-5, s), 98.8 (C-6, d), 165.1 (C-7, s), 93.7 (C-8, d), 156.4 (C-9, s), 103.8 (C-10, s), 120.8 (C-1 , s), 130.8 (C-2 , 6 , d), 115.0 (C-3 , 5 , d), 159.9 (C-4 , s), 101.1 (C-1 , d), 74.0 (C-2 , d), 76.2 (C-3 , d), 70.0 (C-4 , d), 74.1 (C-5 , d), 62.7 (C-6 , t), 125.3 (C-1 , s), 132.6 (C-2 , 6 , d), 114.8 (C-3 , 5 , d), 158.8 (C-4 , s), 143.6 (C-7 , d), 114.6 (C-8 , d), 165.8 (C-9 , s). These spectroscopic data are consistent with those reported in the literature (Chen et al., 2012). Therefore, compound 4 was identified as kaempferol-3-O-(6 -O-*cis*-p-coumaroyl)-β-D-glucopyranoside.

Compound 5 was obtained as a yellow amorphous powder. HR-ESI-MS m/z: 595.1391 $[M+H]^+$, molecular formula $C_{30}H_{26}O_{13}$. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.99 (2H, d, J = 8.0 Hz, H-2 , 6), 7.37 (2H, d, J = 8.5 Hz, H-2 , 6), 6.85 (2H, d, J = 8.0 Hz, H-3 , 5), 6.79 (2H, d, J = 8.5 Hz, H-3 , 5), 6.38 (1H, s, H-8), 6.15 (1H, s, H-6), 6.11 (1H, d, J = 16.0 Hz, H-8), 5.45 (1H, d, J = 7.5 Hz, H-1); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 156.8 (C-2, s), 133.5 (C-3, s), 177.8 (C-4, s), 161.6 (C-5, s), 99.4 (C-6, d), 165.2 (C-7, s), 94.2 (C-8, d), 156.8 (C-9, s), 104.2 (C-10, s), 121.2 (C-1 , s), 131.3 (C-2 , 6 , d), 115.6 (C-3 , 5 , d), 160.5 (C-4 , s), 101.6 (C-1 , d), 74.6 (C-2 , d), 76.7 (C-3 , d), 70.5 (C-4 ,

d), 74.7 (C-5 , d), 63.4 (C-6 , t), 125.4 (C-1 , s), 130.6 (C-2 , 6 , d), 116.2 (C-3 , 5 , d), 160.3 (C-4 , s), 145.1 (C-7 , d), 114.1 (C-8 , d), 166.6 (C-9 , s). These data are consistent with those reported in the literature (Chen et al., 2012). Therefore, compound 5 was identified as kaempferol-3-O-(6 -O-*trans*-p-coumaroyl)-β-D-glucopyranoside.

Compound 6 was obtained as a yellow granular powder. HR-ESI-MS m/z: 595.1792 $[M+H]^+$, molecular formula $C_{30}H_{26}O_{13}$. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.59 (1H, br s, OH-5), 10.18 (1H, br s, OH-4), 8.04 (2H, d, J = 8.5 Hz, H-2 , 6), 7.37 (1H, d, J = 16.5 Hz, H-7), 7.33 (2H, d, J = 8.0 Hz, H-2 , 6), 6.85 (2H, d, J = 8.5 Hz, H-3 , 5), 6.77 (2H, d, J = 8.0 Hz, H-3 , 5), 6.39 (1H, s, H-8), 6.14 (1H, s, H-6), 6.10 (1H, d, J = 16.5 Hz, H-8), 5.45 (1H, d, J = 7.0 Hz, H-1); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 156.3 (C-2, s), 133.2 (C-3, s), 177.0 (C-4, s), 161.1 (C-5, s), 98.8 (C-6, d), 164.4 (C-7, s), 93.7 (C-8, d), 156.3 (C-9, s), 103.7 (C-10, s), 120.8 (C-1 , s), 130.9 (C-2 , 6 , d), 115.7 (C-3 , 5 , d), 160.0 (C-4 , s), 101.6 (C-1 , d), 73.0 (C-2 , d), 71.0 (C-3 , d), 68.2 (C-4 , d), 72.8 (C-5 , d), 63.2 (C-6 , t), 124.9 (C-1 , s), 130.1 (C-2 , 6 , d), 115.0 (C-3 , 5 , d), 159.8 (C-4 , s), 144.6 (C-7 , d), 113.6 (C-8 , d), 166.1 (C-9 , s). These spectroscopic data are consistent with those reported in the literature (Wang et al., 2004). Therefore, compound 6 was identified as castanoside A.

Compound 7 was obtained as a white powder. HR-ESI-MS m/z: 431.3462 $[M+H]^+$, molecular formula $C_{28}H_{46}O_3$. ^{1}H-NMR (500 MHz, C_5D_5N): δ 5.24 (1H, dd, J = 7.5, 15.5 Hz, H-23), 5.17 (1H, dd, J = 8.0, 15.5 Hz, H-22), 4.84 (1H, m, H-3), 4.33 (1H, br s, H-6), 1.54 (3H, s, H-19), 1.07 (3H, d, J = 6.5 Hz, H-21), 0.96 (3H, d, J = 7.0 Hz, H-28), 0.88 (3H, d, J = 8.5 Hz, H-27), 0.86 (3H, d, J = 6.0 Hz, H-26), 0.68 (3H, s, H-18); ^{13}C-NMR (125 MHz, C_5D_5N): δ 32.6 (C-1, t), 33.8 (C-2, t), 67.5 (C-3, d), 41.9 (C-4, t), 76.1 (C-5, s), 74.2 (C-6, d), 120.4 (C-7, d), 141.5 (C-8, s), 43.7 (C-9, d), 38.0 (C-10, s), 22.3 (C-11, t), 39.8 (C-12, t), 43.7 (C-13, s), 55.2 (C-14, d), 23.4 (C-15, t), 28.4 (C-16, t), 56.1 (C-17, d), 12.4 (C-18, q), 18.7 (C-19, q), 40.8 (C-20, d), 20.1 (C-21, q), 136.1 (C-22, d), 132.1 (C-23, d), 43.0 (C-24, d), 33.3 (C-25, d), 21.3 (C-26, q), 19.8 (C-27, q), 17.8 (C-28, q). These data are consistent with those reported in the literature (GAO et al., 2001). Therefore, compound 7 was identified as cerevisterol.

Compound 8 was obtained as a white amorphous powder. HR-ESI-MS m/z: 111.0277 $[M-H]^-$, molecular formula $C_4H_4N_2O_2$. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.39 (1H, d, J = 7.5 Hz, H-6), 5.46 (1H, d, J = 7.5 Hz, H-5); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 151.5 (C-2, s), 164.3 (C-4, s), 100.2 (C-5, d), 142.1 (C-6, d). These spectroscopic data are consistent with those reported in the literature (Gao et al., 2001). Therefore, compound 8 was identified as uracil.

According to literature records, the leaves of *C. lachynocarpus* are used as an ingredient in compound preparations. A traditional Chinese medicine powder formulation known as Zhanjiang Snake Medicine (also called He Xiaosheng Snake Medicine), which combines *C. lachynocarpus* leaves with other medicinal herbs such as *Croton crassifolius* and *Hypericum japonicum*, exhibits swelling reduction, analgesic, and snake venom detoxification effects

(Editing Department of Journal of Snake, 1990). However, to date, no studies have reported on the chemical constituents of the twigs and leaves of *C. lachynocarpus*. This study isolated and identified eight compounds from this plant material, encompassing structural types including clerodane diterpenoids, flavonoids, and sterols. Modern pharmacological research has demonstrated that clerodane diterpenoids generally possess significant anti-inflammatory, antibacterial, and antitumor activities (Chen et al., 2017). The flavonoids kaempferol-3-O-(6 -O-*cis*-p-coumaroyl)-β-D-glucopyranoside (4) and kaempferol-3-O-(6 -O-*trans*-p-coumaroyl)-β-D-glucopyranoside (5) exhibit low cytotoxicity and varying degrees of antioxidant activity, with compound 4 also showing notable anti-inflammatory activity (Ren et al., 2016). Castanoside A (6) demonstrates certain cytotoxic effects (IC_{50} = 77.91 M) (Zhang, 2010). The sterol cerevisterol (7) can inhibit serum starvation-induced apoptosis in MC3T3-E1 cells (Hata et al., 2002). Therefore, this chemical investigation of *C. lachynocarpus* twigs and leaves not only enriches our understanding of the plant' s chemical composition but also provides a scientific basis for its future development and utilization.

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Note: Figure translations are in progress. See original paper for figures.