Study on Chemical Constituents from Tobacco Leaves

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Keywords: Nicotiana tabacum L, Scopoletin, Stigmasterol-3-O-glucopyranoside.

Abstract. [Objective] To study the chemical constituents from tobacco leaves. [Method] The constituents were isolated and purified by silica gel and TLC. Their structures were elucidated by spectroscopy. [Result] Eight compounds were isolated and identified as tetracosane(I), 4-hydroxyphenethyl icosanoate(II), β-sitosterol(III), stigmasterol(IV), scopolin(V), stigmasterol-3-O-glucopyranoside (VI), solanesol(VII), scopoletin(VIII). [Conclusion] Compounds I-VI were isolated from this plant for the first time.

Introduction

Nicotiana tabacum L. is an economic crop widely distributed in world, especially in America, Australia and South Pacific Islands. China is a big country of tobacco growing, with an annual output of about 2.4×10⁶t, widely distributed in Henan, Guizhou and Yunnan provinces. The former was isolated from tobacco leaves, such as alkaloids, proteins, sugars, organic acids and other substances, which have a high economic and medicinal value. As part of our studies on tobacco leaves from Henan province, eight compounds were isolated from this plant and identified as tetracosane (1), 4-hydroxyphenethyl icosanoate (2), β-sitosterol (3), stigmasterol (4), scopolin (5) and stigmasterol-3-O-glucopyranoside (6), solanesol(7), scopoletin(8). Compounds 1~6 were isolated from this plant for the first time.

Experimental

General Procedure

NMR spectra were recorded on the INOVA-400 spectrometer (¹H: 400 MHz and ¹³C: 100 MHz) with TMS as internal standard. Melting points were determined on a X4 apparatus and are not uncorrected. IR spectra were carried out on a Nexus 670 FT-IR spectrophotometer. Silica gel (200~300 mesh) from Qingdao Haiyang Chem. Ind. Co. Ltd. was used for column chromatography (CC). AB-8 macroporous adsorption resin was obtained from Nankai University Chemical Plant (Tianjin, China), all solvents were industrial products, and redistilled before using.

Extraction and Isolation

THE powdered dried defatted seeds of tobacco leaves. (5000 g) were soaked three times with 90% EtOH at room temperature (each 72 h). After evaporating the solvent, 250 g residue was obtained. The extract 80g separated by column chromatography, respectively with petroleum ether and methanol solvent gradient elution. The detection of TLC, with the same components, after repeated by silica gel column chromatography and preparative TLC method to get compound I (1200mg), II (21mg), III (168mg), IV (8mg), V (5mg), VI (120mg) ,VII(56mg), VIII(12mg).

Identification

Compound I: White crystal, mp 48~50 °C. IR(KBr)νmax: 2956, 2917, 2848, 1463, 1378, 720 cm⁻¹. ¹H-NMR(400 MHz, CDCl₃): 1.23 (44H, m, H-2~23), 0.86(6H,t, H-1, 24).
**Compound I:** White powder, mp 80 ~ 82 °C. ESI-MS m/z:433[M+H]+. 

**1H-NMR(400MHz,CDCl3):** δ: 0.85(3H,t,J=6.0 Hz, H-20), 1.22(34H,m,H-4~19), 1.48 (2H,m,H-3), 2.25(2H,t, J=8.0 Hz,H-2), 2.83 (2H, t,J= 7.2 Hz,H-6'), 4.21 (2H,t, J=6.8Hz,H-5'), 6.74(2H,d,J=8.8Hz,H-2'), 7.06(2H, d, J= 8.4 Hz,H-3'). 

**13C–NMR (100MHz,CDCl3):** 173.9(C=O), 154.2(C-1'), 130.0(C-3', 4'), 115.3(C-2'), 64.9 (C-6'), 50.1(C-9), 36.8(C-10), 20.0(C-11), 39.7(C-12), 42.3(C-13), 56.8(C-14), 24.3(C-15), 28.9(C-16), 56.0(C-17), 12.2(C-18), 19.4(C-19), 40.5(C-20), 21.2(C-21), 138.0(C-22), 129.0(C-23), 51.2(C-24), 31.9 (C-25), 19.0(C-26), 21.2(C-27), 25.4(C-28), 11.8(C-29). 

**Compound III:** Colorless needles (Trichloromethane), mp 136~138 ℃. IR(KBr)ν max: 3430, 2960, 2936, 2890, 1465, 1380, 1065, 960 cm-1. Liebermann-Burchard positive reaction, Molish negative reaction, TLC in case of 5% sulfuric acid ethanol significantly purple red. 

**Compound IV:** Colorless crystal (Trichloromethane), mp 147~149 ℃. TLC in the case of sulfuric acid ethanol significantly purple red. ESI-MS m/z:413[M+H]+. 

**13C-NMR (400 MHz, DMSO-d6) δ: 37.2(C-1), 31.6(C-2), 71.7(C-3), 42.6(C-4), 140.7(C-5), 121.7(C-6), 31.8(C-7), 31.9(C-8), 50.1(C-9), 36.4(C-10), 20.0(C-11), 39.7(C-12), 42.3(C-13), 56.8(C-14), 24.3(C-15), 28.9(C-16), 56.0(C-17), 12.2(C-18), 19.4(C-19), 40.5(C-20), 21.2(C-21), 138.0(C-22), 129.0(C-23), 51.2(C-24), 31.9 (C-25), 19.0(C-26), 21.2(C-27), 25.4(C-28), 11.8(C-29). 

**Compound V:** Colorless needles (DMSO), mp 220~222 ℃. IR(KBr)ν max: 3411 (-OH), 2934, 2870, 1630, 1462, 1367, 1074, 1025, 796 cm⁻¹. 

**1H-NMR (400 MHz, DMSO-d6):** δ: 3.90(3H,s,OCH₃), 6.27(1H,d,J=9.2Hz,H-3), 6.82(1H,s,H-8), 6.89(1H,s,H-5), 7.57(1H,d,J=9.2 Hz, H-4). 

**13C-NMR (400 MHz, DMSO-d6) δ: 161.4(C-2),149.9(C-9),149.6(C-7),143.9(C-6), 143.3(C-4), 113.4(C-3), 111.4 (C -10), 107.4(C-5), 103.0(C-8),56.3(OCH₃),99.4(C-1'), 72.8(C-2'),76.3(C-3'), 68.2(C-4'), 75.7(C-5'),59.4(C-6'). NMR date was the same as those of stigmasterol-3-O-glucopyranoside. 

**Compound VI:** Colorless needles (DMSO), mp 277~281 ℃. ESI-MS m/z:631[M+H]+. 

**1H-NMR (400 MHz, CDCl₃)δ: 5.12(8H,m, H-2, 6, 10, 14, 18, 22, 26, 30), 4.13(2H, d, H-1), 2.11(16H,m, H-5, 9, 13, 17, 21, 25, 29, 33), 2.02(1H, s, OH), 1.98(16H,t, H-4,8,12,16,20,24,28,32),1.70(6H,s, H-36,37),1.58(24Hs , H-38~45). 

**13C-NMR (400 MHz, CDCl₃):** δ: 139.3(C-3),136.5(C-2), 135.1(C-7,11,15,19,23,27,31),124.3(C-6,10,14,18,22,26,30),123.9(C-34),122.4(C-35),16.6(C-36,37),16.4(C-38~45).MS, NMR date were the same as those of solanesol[2]. 

**Compound VII:** Yellow crystal, mp 41~43 ℃. ESI-MS m/z:631[M+H]+. 

**1H-NMR (400 MHz, CDCl₃):** δ: 5.12(8H,m, H−2, 6, 10, 14, 18, 22, 26, 30), 4.13(2H, d, H-1), 2.11(16H,m, H-5, 9, 13, 17, 21, 25, 29, 33), 2.02(1H, s, OH), 1.98(16H,t, H-4,8,12,16,20,24,28,32),1.70(6H,s, H-36,37),1.58(24Hs , H-38~45). 

**13C-NMR (400 MHz, CDCl₃):** δ: 139.3(C-3),136.5(C-2), 135.1(C-7,11,15,19,23,27,31),124.3(C-6,10,14,18,22,26,30),123.9(C-34),122.4(C-35),16.6(C-36,37),16.4(C-38~45).MS, NMR date were the same as those of solanesol[2].

**Compound VIII:** Colorless needles, mp 200~202 ℃. ESI-MS m/z:193[M+H]+. 

**1H-NMR (400 MHz, CDCl₃):** δ: 3.93(3H,s, OCH₃),6.24(1H,d,J=9.2 Hz,H-3), 6.82(1H,s, H -8), 6.89 (1H,s,H-5), 7.58(1H,d,J=9.2 Hz,H-4). 

**13C-NMR (400 MHz, CDCl₃):** δ: 161.4(C-2), 15.2 (C -9), 149.6(C-7), 143.9(C-6), 143.3(C-4), 113.4(C-3), 111.4 (C -10), 107.4(C-5), 103.1(C-8), 56.4 (OCH₃). NMR date was the same as those of scopoletin.
References
