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Identification of chemical constituents of the seed crusts from *Trewia nudiflora* L.

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Abstract: **Aim** To study the chemical constituents of the seed crusts from *Trewia nudiflora* L. **Method** Five compounds were confirmed by the physical and chemical properties and the data of spectra. **Result and conclusion** The structures of the five compounds were isolated from the seed crusts of *Trewia nudiflora* and determined to be 2,3-dihydro-3,6,7-trihydroxy-1-*H*-benzo[b]pyran-4-one(**1**), α,2′,4,4′-tetrahydroxydidrochalaone(**2**), isoliquiritigenin(**3**), 3-(3,4-dihydroxyphenyl)-prop-2-en-1-ol(**4**), β-sitosterol(**5**). Among those, compound **1** was determined to be a new compound, named nudiflone. Compound **3** showed antibacterial activity.

Key words: medicinal chemistry; isolation; identification; chromatography; *Trewia nudiflora* L.; nudifone
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Antimicrobial resistance is virtually emerging in all the nosocomial pathogen-antimicrobial combinations, which highlights the need to stimulate further research on strategies aimed at preserving the effectiveness of currently available antibacterial agents and finding new classes of antibacterial agents. Natural products produced by higher plants evolved from selection for acquisition of the improved defense against microbial attacks[1], therefore, are less likely to incur resistance.

The genus *Trewia* (Euphorbiaceae) included only one species, spread in India, Malaysia and China. Some maytansinoids isolated from the *Trewia nudiflora* seeds were tumor inhibitors[2,3]. The seeds of *T. nudiflora* could be stored for several years under normal conditions, so the seed crusts of *T. nudiflora* must be resistant to soil microorganisms and fast rot. However, there was no report about the isolation of antibacterial components from the seeds of *T. nudiflora*, especially from the seed crusts. The isolation, structure identification and the antibacterial activities against gram-positive bacterium *Staphylococcus aureus* and gram-negative bacterium *Mycobacterium tuberculosis* of the chemical constituents of the seed crusts of *T. nudiflora* are reported here.

1 Experiment

Optical rotations were measured on a JASCO DIP - 370 Digital Polarimeter. The IR spectra were measured on a Perkin-Dlmer - 577 spectrophotometer. UV spectra were obtained on a Shimadzu double-beam 210A spectrophotometer. The NMR spectra were recorded on Brucker DRX - 500 spectrometers. MS were performed on a VG AutoSpec-3000 spectrometer. TLC was performed on plates precoated with silica gel

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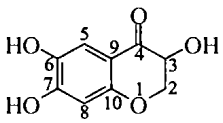
(Qingdao Marine Chemical Co., Ltd., The People's Republic of China). Reversed-phase (RP) C_{18} silica gel for column chromatography was obtained from Merck. Sephadex LH-20 for column chromatography was purchased from Amersham Biosciences. The solvents were distilled before using.

1.1 Extraction and isolation

The seeds of *T. nudiflora* were collected at Xishuangbanna, Yunnan Province, The People's Republic of China, in December 1998. The seed crusts of *T. nudiflora* (dry weight: 6.5 kg) were extracted three times with 70% (φ) ethanol. The ethanol extract was portioned between acetyl acetate and water. The acetyl acetate extract was extracted with petroleum ether (PE) and chloroform (CH) to produce two fractions: PE and CH. Fraction CH (105 g) was chromatographed on silica gel, RP-18 and Sephadex LH-20 column to produce compounds **1** (11 mg), **2** (3 mg), **3** (5 mg), **4** (42 mg). The fraction PE (152 g) was chromatographed on silica gel column and recrystallized from acetone to afford compound **5** (200 mg).



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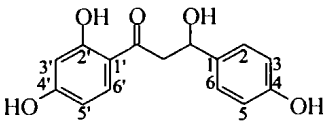

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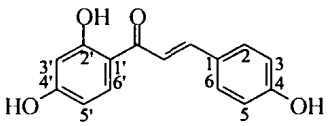

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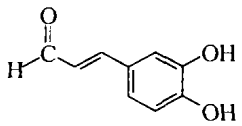

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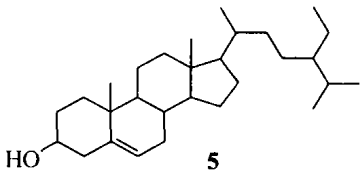

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1.2 Physical and spectral data

Compound **1**, yellow powder, $[\alpha]_D^{23}$ − 8.0° (*c* 0.50, MeOH). UV (MeOH) λ_{max}: 210, 238, 279, 340 nm. EIMS *m/z* (%): 196 $[M]^+$ (66), 152 (100), 124(28), 82(43). IR (KBr) σ: 3406, 1664, 1622, 1586, 1518, 1477, 1454, 1289, 1167 cm^{-1}. see ^1H- and ^{13}C-NMR data in Table 1.

Table 1 The NMR data of compound 1 in CD_3OD

Position	^{13}C	^1H	HMBC
2	72.3	4.73(dd, *J* = 5.3, 10.5 Hz)	69.9, 158.1, 193.1
		4.45[t(dd), *J* = 10.7 Hz]	69.9, 158.1(w), 193.1
3	69.9	4.78(dd, *J* = 5.2, 10.7 Hz)	72.3, 158.1, 193.1
4	193.1		
9	112.5		
5	111.9	7.91(s)	143.2, 156.4, 158.1, 193.1, 104.3(w)
6	143.2		
7	156.4		
8	104.3	6.88(s)	112.5, 143.2, 158.1, 156.4
10	158.1		

Compound **2**, yellow needle (MeOH). EIMS *m/z* (%): 256 $[M - H_2O]^+$ (78), 239 (10), 163 (25), 137 (100), 120 (67), 91 (19). ^1H-NMR (400 MHz, MeOD) δ: 2.71 (1H, d, *J* = 16.9 Hz), 3.08 (1H, t, *J* = 13.1 Hz), 6.35 (1H, s, H-3′), 6.50 (1H, dd, *J* = 8.7, 2.1 Hz, H-2), 6.81 (2H, d, *J* = 8.9 Hz, H-2, H-6), 7.32 (2H, d, *J* = 8.9 Hz, H-3, H-5), 7.73 (1H, d, *J* = 8.7 Hz, H-6′). Those data were consistent with literature[4], therefore, compound **2** was determined to be α, 2′, 4, 4′-tetrahydroxydihydrochalcone.

Compound **3**, yellow oil. EIMS *m/z* (%): 256

$[M]^+$ (100), 255 (75), 239 (17), 192 (4), 150 (25), 163 (41), 137 (93), 120 (42), 91 (22), 77 (6). Those data were consistent with literature[5], therefore, compound **3** was determined to be isoliquiritigenin.

Compound **4**, yellow crystal (MeOH). EIMS m/z (%): 164 $[M]^+$ (100), 147 (49), 136 (25), 77 (17). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.57 (1H, dd, J = 15.6, 10.1 Hz, H-8), 6.82 (1H, d, J = 8.2 Hz, H-5), 7.05 (1H, dd, J = 8.2, 2.1 Hz, H-6), 7.12 (1H, d, J = 2.1 Hz, H-2), 7.54 (1H, d, J = 15.7 Hz, H-7), 9.56 (1H, d, J = 11.9 Hz, H-9). Those data were very much characteristic of caffeic esters[6], so compound **4** was determined to be 3-(3, 4-dihydroxyphenyl)-prop-2-en-1-ol.

Compound **5**, colorless needle (acetone). EIMS m/z (%): 414 $[M]^+$ (100), 396 (M − H_2O, 27), 381 (18), 367 (37), 354 (5), 329 (20), 303 (34), 289 (5), 273 (15), 255 (15), 231 (12), 213 (16), 145 (17), 109 (14) and 69 (25). Comparing with the standard sample on TLC over silica gel, compound **5** was determined to be β-sitosterol.

1.3 Antibacterial activity

The screening of compounds **1**～**4** for the antibacterial activities was assayed against *Staphylococcus aureus* and *Mycobacterium tuberculosis* using the paper disc diffusion method[7]. The results showed that compound **3** had modest inhibitory activities against *S. aureus* and *M. tuberculosis* (Table 2).

Table 2 Antimicrobial activities of compounds 1～4 and controls mm

Test organisms	Compounds (100 μg/disk)				Controls		
	1	2	3	4	carbenicillin sodium (10 μg/disk)	streptomycin sulfate (10 μg/disk)	rifampicin (0.2 μg/disk)
M. tuberculosis	−	−	6.5	−	15.0	11.0	15.0
S. aureus	−	−	6.5	−	12.0	10.0	15.0

Note: The data are the mean of the three replicates

2 Result and discussion

Compound **1**, the HRESIMS indicated the molecular formula to be $C_9H_8O_5$ (m/z 219.0262 $[M + Na]^+$, calcd. 219.0269). The IR spectra showed that **1** had a benzene ring (1586, 1477, 1518 cm^{-1}), which was supported by the UV spectra (λ_{max} 238 nm). The spectra of ^{1}H- and ^{13}C-NMR data showed that **1** had one methylene, three methines and five quaternary carbons, including one carbonyl group, indicating the presence of a symmetric four-substituted benzene ring [δ_C: 158.1 (s), 156.4 (s), 143.2 (s), 112.5 (s), 111.9 (d), 104.3 (d); δ_H: 7.91 (s), 6.88 (s)]. The conjugate effect which was observed in the NMR with the chemical shift at δ_C: 193.1 (s) and IR absorption at 1664 cm^{-1} indicating the linkage of the carbonyl functional group with the benzene ring. The structure of the pyranone was elucidated based on the HMBC between the methylene protons at δ 4.73 (H-2) and the carbon at δ 69.9 (C-3), 158.1 (C-10) and 193.1 (C-4), and the correlations between the methine proton at δ 4.78 (H-3) and the carbons at δ 72.3 (C-2), 158.1 (C-10) and 193.1 (C-4). Therefore, compound **1** was determined to be 2, 3-dihydro-3, 6, 7-trihydroxy-1-*H*-benzo [b] pyran-4-one, a new compound named nudiflone.

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滑桃树种壳化学成分的鉴定

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摘 要:目的 研究滑桃树种壳中的化学成分。**方法** 通过理化性质和光谱数据的分析鉴定了5个化合物的结构。**结果与结论** 从滑桃树(*Trewia nudiflora* L.)种壳中分离到5个化合物。分别鉴定为:2,3-二氢-3,6,7-三羟基-1-苯丙吡喃-4-酮(**1**)、α,2′,4,4′-tetrahydroxydidrochalaone(**2**)、isoliquiritigenin(**3**)、3,4-二羟基桂皮醛(**4**)和β-谷甾醇(**5**),其中化合物**1**是新化合物。化合物**3**具有一定的抗细菌活性。

关键词:药物化学;分离;鉴定;色谱分析;滑桃;2,3-二氢-3,6,7-三羟基-1-苯丙吡喃-4-酮