Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

A new phenolic glycoside and two new monoterpenoid furocoumarins from Aurantii Fructus Immaturus

Ying Xiong, Meiyan Chang, Kezhong Deng & Yongming Luo

To cite this article: Ying Xiong, Meiyan Chang, Kezhong Deng & Yongming Luo (2016): A new phenolic glycoside and two new monoterpenoid furocoumarins from Aurantii Fructus Immaturus, Natural Product Research, DOI: 10.1080/14786419.2015.1118634

To link to this article: http://dx.doi.org/10.1080/14786419.2015.1118634

NATURAL PRODUCT RESEARCH, 2016
http://dx.doi.org/10.1080/14786419.2015.1118634

Taylor & Francis
Taylor & Francis Group

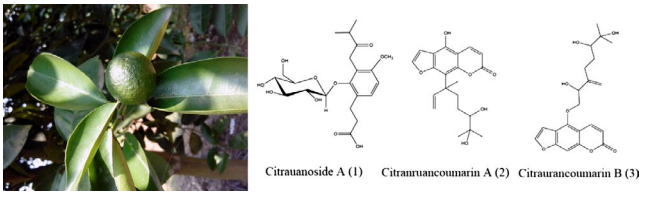
A new phenolic glycoside and two new monoterpenoid furocoumarins from Aurantii Fructus Immaturus

Ying Xiong, Meiyan Chang, Kezhong Deng and Yongming Luo

College of Pharmacy, Jiangxi University of Traditional Chinese Medicine, Nanchang, China

ABSTRACT

A new phenolic glycoside, citrauranoside A (**1**), and two new monoterpenoid furocoumarins, citraurancoumarin A (**2**) and citraurancoumarin B (**3**), along with four known compounds (**4–7**) were isolated from the young fruit of *Citrus aurantium* L. The structures were elucidated by their comprehensive analysis including 1D, 2DNMR, IR and mass spectra.



ARTICLE HISTORY
Received 13 August 2015
Accepted 10 October 2015

KEYWORDS
Aurantii Fructus Immaturus; coumarin; phenolic glycoside

1. Introduction

Aurantii Fructus Immaturus is derived from the young fruit of *Citrus aurantium* L. and its cultivated varieties or *Citrus sinensis* Osbeck (Rutaceae) which is mainly produced in the province of Jiangxi, Hunan and Sichuan. It has been used for treatment of food retention syndromes, chest impediment and epigastric stuffiness in Traditional Chinese Medicine. Previous phytochemical investigations have reported flavonoids, coumarins (Zhang et al. 2005; Feng et al. 2012; Wen et al. 2014), limonoids, alkaloids (Zhang et al. 2015), chromone (Jiang et al. 2015) and essential oils in this plant. This article deals with the isolation and structural determination of seven compounds from the young fruit of *C. aurantium* L., including a novel phenolic glycoside (**1**) and two new monoterpenoid furancoumarins (**2**,**3**) together with two known coumarins (**4**,**5**) and two known benzene derivatives (**6**,**7**).

2. Results and discussion

The ethyl acetate soluble fraction of ethanol extract of the young fruit of *C. aurantium* L. was purified by column chromatography (silica gel, ODS, Sephadex LH-20 and HPLC) to afford four coumarins and three benzene derivatives, including three new compounds (**1–3**) (Figure 1).

CONTACT Kezhong Deng dengkezhong@jxutcm.edu.cn
The supplementary material for this paper is available online at http://dx.doi.org/10.1080/14786419.2015.1118634.

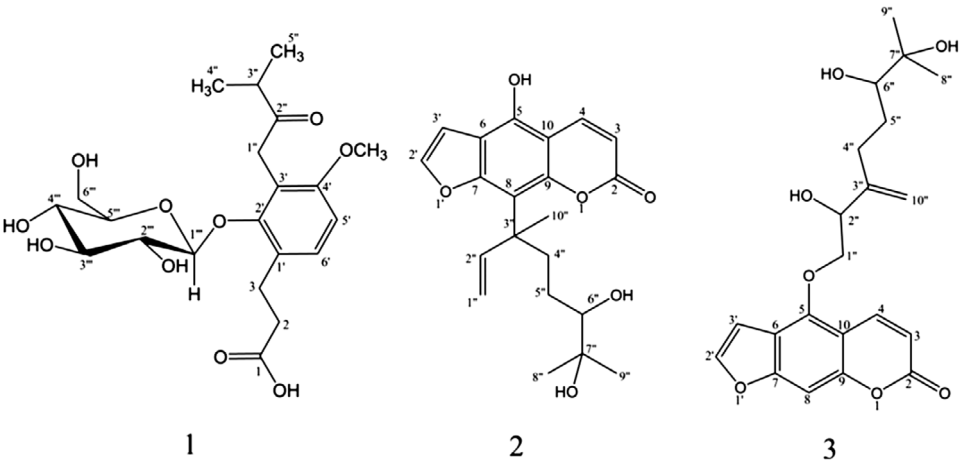


Figure 1. The structures of compounds **1–3**.

Compound **1**, a white amorphous powder, had the molecular formula of $C_{21}H_{30}O_{10}$ deduced from the positive HRESIMS for the peak at *m/z* 460.2186, $[M + NH_4]^+$, Calcd 460.2183. The IR spectrum exhibited absorption bands at 3436, 1709, 1609 and 1562 cm^{-1} for hydroxyl group, carbonyl group and benzene ring system, respectively. The ^1H NMR spectrum of **1** exhibited signals characteristic for an isopropyl group[δ_H 2.82 (1H, m), δ_H 1.15 (3H, d, *J* = 7.2 Hz), δ_H 1.14 (3H, d, *J* = 7.2 Hz)], a methylene [δ_H 4.00 (1H, d, *J* = 17.6 Hz), 4.18 (1H, d, *J* = 17.6 Hz)], a methoxyl [δ_H 3.72 (3H, s)] and a 1,2,3,4-tetrasubstituted aromatic ring[δ_H 7.21 (1H, d, *J* = 8.4 Hz), 6.72 (1H, d, *J* = 8.4 Hz)]. The ^1H and ^{13}C NMR spectra at δ_H 4.56 (1H, d, *J* = 7.6 Hz), 3.46 (1H, t, *J* = 8.0 Hz), 3.37 (2H, m), 3.11 (1H, m), 3.77 (1H, dd, *J* = 12.0, 2.5 Hz), 3.63 (1H, dd, *J* = 12.0, 5.2 Hz) and δ_C 105.1, 76.8, 76.7, 74.1, 70.0 and 61.2 suggested the presence of one beta glucose moiety, In addition, the sugar was also determined as D-glucose by the acid hydrolysis and then comparison with authentic sample on TLC. The glucopyranose moiety was determined to have a β-configuration, supported by the coupling constant of the glucose anomeric proton H-1‴(*J* = 7.6 Hz). Correlations from H-1‴ (δ_H 4.56) to C-2′ (δ_C 154.1) in the HMBC spectrum indicated that the β-glucopyranose group was located at C-2′. The isopropyl group was linked with benzyl group through a carbonyl, while the methoxyl was connected to C-4′, as evidence by the HMBC correlations from H-3″, H-4″ and H-5″ to C-2″, from H-1″ to C-2′, C-3′, C-4′, C-2″ and from methoxy protons to C-4′. Furthermore, a propionyloxy (δ_C 177.5, 35.7, 25.3) was linked to C-1′ confirmed by HMBC [between H-2,H-3 and C-1′]. The structure of compound **1** was therefore determined as 3-[4-methoxy-3-(3-methyl-2-oxobutyl)-2-O-β-D-glucopyranosyl-phenyl]propanoic acid, named citrauranoside A.

Compound **2** was obtained as a faint yellow powder. Its molecular formula, $C_{21}H_{24}O_{6}$, was deduced from its positive HRESIMS (*m/z* 373.1643, $[M + H]^+$ Calcd 373.1646) and NMR data analysis. The IR spectrum showed absorption bands at 3434, 1700 and 1617 cm^{-1}, indicating the presence of hydroxyl group, carbonyl group and aromatic ring system, respectively. In the ^1H NMR and ^{13}C NMR spectra of **2**, two pairs of one-proton doublets [δ_H 6.18 (1H,d, *J* = 9.6 Hz), 8.34 (1H, d, *J* = 9.6 Hz); δ_H 7.68 (1H, d, *J* = 2.0 Hz),7.04 (1H, d, *J* = 2.0 Hz)] in conjunction with δ_C 162.3, 157.1, 150.7, 147.0, 143.8, 140.8, 113.7, 109.9, 109.5, 105.0, 103.3 were indicative of a 3,4-unsubstituted furanocoumarin. The remaining 10 carbon signals together

with HSQC experiments suggested the presence of three methyl singlets [δ_H 1.83 (3H, s), δ_C 26.2; δ_H 1.03 (3H, s), δ_C 24.3; δ_H 0.98 (3H, s), δ_C 23.8], two methylenes [δ_H 2.64 (1H, m), 2.02 (1H, m), δ_C 38.2; δ_H 1.31 (1H, m), 1.13 (1H, m), δ_C 26.9], two quaternary carbons (δ_C 44.5 and 72.5), one oxymethine [δ_H 3.23 (1H, m), δ_C 79.2] together with one double bond [δ_H 6.47 (1H, dd, J = 10.8, 17.6 Hz),5.00 (1H, br d, J = 17.6 Hz), 4.93 (1H, br d, J = 10.8 Hz) and δ_C 147.7, 109.3]. A myrcenyl moiety, 6,7-dihydroxy-3,7-dimethyloct-1-en-3-yl, in the molecule was deduced from the ^{1}H-^{1}H COSY [between H-4″ and H-5″] and HMBC [between H-1″,2″,4″,10″ and C-3″ (δ_C 44.5); the correlation of H-5″,8″,9″ with both C-6″ (δ_C 79.2) and C-7″ (δ_C 72.5)]. Furthermore, the HMBC correlations between H-2″,4″,10″ and C-8 (δ_C 109.9) displayed that the substituted group and furocoumarin backbone were linked via C-3″-C8. Thus, based on the above evidence, compound **1** was established as 8-(6,7-dihydroxy-3,7-dimethyloct-1-en-3-yl)-5-hydroxy-6,7-furocoumarin, named citraurancoumarin A.

Compound **3**, isolated as white amorphous powder, was determined to have a molecular formula of $C_{21}H_{24}O_7$ by its HRESIMS (*m/z* 389.1590, $[M + H]^+$ Calcd 389.1595). The IR spectrum suggested the presence of hydroxyl (3431 cm^{-1}), carbonyl (1721 cm^{-1}) and phenyl groups (1625, 1578 cm^{-1}). Analysis of the ^{1}H and ^{13}C NMR spectra indicated compounds 3 and 2 had the same furocoumarin skeleton, but the different side chain. In the NMR spectrum for the side chain, instead of three olefinic proton signals at δ_H 6.47, 5.00 and 4.93 and one methyl singlets at δ_H 1.83 in 2, five signals due to oxymethine, oxymethylene and olefinic methylene appeared at δ_H 4.57 (1H, m), δ_H 4.41 (2H, m) and δ_H 5.26 (1H,br d, J = 11.2 Hz), 5.07 (1H,br s) respectively, in the ^{1}H NMR spectrum of 3. The HMBC correlations of H-4 (δ_H 8.15), 1″ (δ_H 4.41) with C-5 (δ_C 148.3) supported this unit connected to C-5. The NMR data of 3 was also very similar to those of praealtin D (Wilzer et al. 1989) except for one more furan ring [δ_H 7.55 (1H, d, J = 2.0 Hz), 6.95 (1H, d, J = 2.0 Hz), δ_C 145.0, 104.5]. Thus, compound **3** was assigned as 5-(2,6,7-trihydroxy-7-methyl-3-octaenyloxy)-6,7-furocoumarin, named citraurancoumarin B.

The stereochemistry of the two new furocoumarin remains to be determined. According to the previous literature, the tentative absolute configuration of C-6″ was determined as R in praealtin D on biogenetic grounds (Wilzer et al. 1989).

Two known coumarins, Praealtin D (**4**) (Wilzer et al. 1989), bergapten (**5**) (Guan et al. 2007) and two known benzene derivatives, 3,5-dihydroxyphenyl-β-D-glucopyranoside (**6**) (Sakar et al. 1993), benzoic acid (**7**) (Yu et al. 2015), were identified by the comparison of their physical and spectroscopic data with literature values.

3. Experimental

3.1. General experimental procedures

IR spectra (KBr) were obtained on a Bio-Rad FTS 6000 infrared spectrometer. HRESIMS spectra were performed on an Ionspec 7.0T FTICR MS. 1D and 2D NMR spectra were recorded on a Bruker AVANCE-400 NMR spectrometer using TMS as internal standard. Preparative HPLC was performed on a CXTH LC-3000 liquid chromatography with an ODS column (YMC-pack ODS-A, 5 μm, 250 × 20 mm). Silica gel (200–300 mesh, 300–400 mesh, Qingdao Ocean Chemical Group Co. of China), Sephadex LH-20 (Merck Co.) and HW-40F (TOSOH) for column chromatography as well as silica gel GF_{254} (Qingdao Ocean Chemical Group Co. of China) for TLC were used.

3.2. Plant material

The young fruit of *C. aurantium* L. were purchased from Zhangshu, Jiangxi province, China, in July of 2013 and was identified by Dr. Ke-Zhong Deng. A voucher specimen (No. ZS13008) is deposited in the College of Pharmacy, Jiangxi University of Traditional Chinese Medicine, China.

3.3. Extraction and isolation

The young fruit of *C. aurantium* L. (50 kg) were powdered and successively extracted with 95 and 75% EtOH by diacolation and filtered. After removal of the solvent under reduced pressure, the residue (12 kg) was suspended in water and partitioned with petroleum ether, ethyl acetate and n-BuOH successively. The EtOAc extract (1 kg) was subjected to silica gel column chromatography (CC, 18 × 150 cm, 200–300 mesh, 5 kg), eluted with CH_2Cl_2/CH_3OH (1:0, 98:2, 97:3, 95:5, 9:1, 8:2, 7:3, 6:4, 1:1, 0:1) to give 29 fractions. Fraction 4（49 g）was chromatographed on silica gel CC (8 × 120 cm, 200–300 mesh, 900 g) eluted with a gradient solvent of petroleum ether/EtOAc (1:0 → 0:1) to obtain nineteen fractions (Fr.4–1-Fr.4–19). Compound **6** (240 mg) and **7** (178 mg) were crystallised from Fr.4–3 and Fr.4–10, respectively. Fraction 15（20 g）was subjected to silica gel CC (6 × 100 cm, 300–400 mesh, 500 g) eluted with a gradient solvent of CH_2Cl_2/CH_3OH (1:0 → 8:2) to obtain 20 fractions (Fr.15–1-Fr.15–20). Fr.15–8 and Fr.15–10 applied to Sephadex LH-20 CC eluted with CH_2Cl_2/CH_3OH (1:1) to obtain compounds **3** (26 mg) and **4** (35 mg), respectively. The further separation of Fr.15–9 by Sephadex LH-20 CC eluted with CH_2Cl_2/CH_3OH (1:1) and preparative TLC (silica gel, thickness 1 mm) with CH_3Cl/CH_3OH (15:1) to give compound **2** (45 mg). Fraction 25 (12 g) was loaded on an open ODS column and eluted with H_2O/MeOH (3:7 → 0:1,v/v) to give 9 subfractions Fr.25–1-Fr.25–9. Fr.25–4 was applied to pre-HPLC (YMC-pack ODS-A, 250 mm × 20 mm, MeOH/H_2O 4:6, 5 ml/min, 210 nm) and purified by Sephadex LH-20 column eluted with MeOH to yield compound **1** (28 mg) and **5** (34 mg).

Citrauranoside A (**1**), $C_{21}H_{30}O_{10}$, white amorphous powder; IR (KBr): 3436, 2969, 2929, 1709, 1709, 1609, 1562, 1489, 1466, 1403, 1385, 1316, 1273, 1201, 1098, 1074, 1046, 896, 808, 576 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 2.57 (2H, t, J = 8.0, H-2), 3.05 (2H, t, J = 8.0, H-3), 6.72 (1H, d, J = 8.4 Hz, H-5′), 7.12 (1H, d, J = 8.4 Hz, H-6′), 4.00 (1H, d, J = 17.6 Hz, H-1″a), 4.18 (1H, d, J = 17.6 Hz, H-1″b), 2.82 (1H, m, H-3″), 1.15 (3H, d, J = 7.2 Hz, H-4″), 1.14 (3H, d, J = 7.2 Hz, H-5″), 4.56 (1H, d, J = 7.6 Hz, H-1‴), 3.46 (1H, t, J = 8.0 Hz, H-2‴),3.37 (2H, m, H-3‴, 4‴), 3.11 (1H, m, H-5‴), 3.77 (1H, dd, J = 12.0, 2.5 Hz, H-6‴a), 3.63 (1H, dd, J = 12.0, 5.2 Hz, H-6‴b), 3.72 (3H, s, OCH_3); ^{13}C NMR (100 MHz, CD_3OD): δ 177.5 (C-1), 35.7 (C-2), 25.3 (C-3), 127.1 (C-1′), 154.1 (C-2′), 118.3 (C-3′), 156.9 (C-4′), 106.9 (C-5′), 128.2 (C-6′), 36.9 (C-1″), 215.4 (C-2″), 40.4 (C-3″), 17.5 (C-4″), 17.4 (C-5″), 105.1 (C-1‴), 74.1 (C-2‴), 76.7 (C-3‴), 70.0 (C-4‴), 76.8 (C-5‴), 61.2 (C-6‴), 54.7 (OCH_3). HRESIMS *m/z* 460.2186 $[M + NH_4]^+$ (Calcd for $C_{21}H_{34}O_{10}N$, 460.2183).

Citraurancoumarin A (**2**), $C_{21}H_{24}O_6$, faint yellow powder; IR (KBr): 3434, 2965, 2926, 1700, 1617, 1467, 1384, 1351, 1266, 1207, 1146, 1078, 827, 753, 728, 574 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 6.18 (1H, d, J = 9.6, H-3), 8.34 (1H, d, J = 9.6, H-4), 7.68 (1H, d, J = 2.0 Hz, H-2′), 7.04 (1H, d, J = 2.0 Hz, H-3′), 5.00 (1H, br d, J = 17.6 Hz, H-1″a), 4.93 (1H, br d, J = 10.8 Hz, H-1″b), 6.47 (1H, dd, J = 10.8, 17.6 Hz, H-2″), 2.64 (1H, m, H-4″a), 2.02 (1H, m, H-4″b), 1.31 (1H, m, H-5″a), 1.13 (1H, m, H-5″b), 3.23 (1H, m, H-6″), 1.03 (3H, s, H-8″), 0.98 (3H, s, H-9″), 1.83 (3H, s, H-10″); ^{13}C NMR (100 MHz, CD_3OD): δ 162.3 (C-2), 109.5 (C-3), 140.8 (C-4), 150.7 (C-5), 113.7

(C-6), 157.1 (C-7), 109.9 (C-8), 147.0 (C-9), 105.0 (C-10), 143.8 (C-2′), 103.3 (C-3′), 109.3 (C-1″), 147.8 (C-2″), 44.5 (C-3″), 38.2 (C-4″), 26.9 (C-5″), 79.2 (C-6″), 72.5 (C-7″), 24.3 (C-8″), 23.8 (C-9″), 26.2 (C-10″). HRESIMS *m/z* 373.1643 $[M + H]^+$ (Calcd for $C_{21}H_{25}O_6$, 373.1646).

Citraurancoumarin B (**3**), $C_{21}H_{24}O_7$, white amorphous powder; IR (KBr): 3431, 2971, 2931, 1721, 1625, 1579, 1458, 1384, 1347, 1284, 1206, 1156, 1134, 1079, 941, 900, 826, 750 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.20 (1H, d, $J = 9.6$, H-3), 8.15 (1H, d, $J = 9.6$, H-4), 7.07 (1H, s, H-8), 7.55 (1H, d, $J = 2.0$ Hz, H-2′), 6.95 (1H, d, $J = 2.0$ Hz, H-3′), 4.41 (2H, m, H-1″), 4.57 (1H, m, H-2″), 2.32 (1H, m, H-4″a), 2.17 (1H, m, H-4″b), 1.70 (1H, m, H-5″a), 1.55 (1H, m, H-5″b), 3.43 (1H, m, H-6″), 1.20 (3H, s, H-8″), 1.15 (3H, s, H-9″), 5.26 (1H, br d, $J = 11.2$ Hz, H-10″a), 5.07 (1H, br s, H-10″b); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.3 (C-2), 112.4 (C-3), 139.4 (C-4), 148.3 (C-5), 113.7 (C-6), 158.0 (C-7), 94.2 (C-8), 152.1 (C-9), 106.9 (C-10), 145.0 (C-2′), 104.5 (C-3′), 75.6 (C-1″), 73.8 (C-2″), 147.3 (C-3″), 28.2 (C-4″), 29.9 (C-5″), 78.0 (C-6″), 73.0 (C-7″), 26.3 (C-8″), 23.1 (C-9″), 113.0 (C-10″). HRESIMS *m/z* $[M + H]^+$ 389.1590 (Calcd for $C_{21}H_{25}O_7$, 389.1595).

3.4. Acidic hydrolysis of 1

Compound **1** (4 mg) was refluxed with 5% HCl (5 ml) in 50% methanol for 2 h. The reaction mixture was neutralised with 2% KOH/H_2O and extracted with ethyl acetate. The R_f value checked by TLC (EtOAc/MeOH/H_2O/acetic acid, 13:4:4:3) and optical activity of the monosaccharide in the residue were identical with those (R_f:5.1; optical activity: positive) of authentic d-glucose.

4. Conclusion

In our study, a novel phenolic glycoside, citrauranoside A (**1**), and two new monoterpenoid furocoumarins, citraurancoumarin A (**2**) and citraurancoumarin B (**3**), as well as four known compounds, praealtin D (**4**), bergapten(**5**), 3,5-dihydroxyphenyl-β-D-glucopyranoside (**6**) and benzoic acid (**7**) were isolated from Aurantii Fructus Immaturus. This is the first report of coumarin possessing a 6,7-dihydroxy-3,7-dimethyloct-1-en-3-yl group from *Citrus* L. Compounds **4**–**7** were isolated from this plant for the first time.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the National Natural Science Foundation of China [grant number 81260621] and the Innovation Fund Designated for Graduate Students of Jiangxi Province [grant number YC2015-S347].

References

Feng F, Wang XN, Yan CM. 2012. Studies on the chemical constituents of *Citrus aurantium* L. Asia-Pacific Tradit Med. 8:22–24.

Guan YX, Yang XS, Tong LH, Yang B, Hao XJ. 2007. Chemical constituents in *Ficus tikoua* of Miao nationality. Chin Tradit Herb Drugs. 38:342–344.

Jiang Z, Liu F, Zhong AJ, Dugu JX, Li XZ. 2015. Determination of a new chromone from Aurantii Fructus Immaturus by DFT/GIAO method. Nat Prod Res. doi:10.1080/14786419.2015.1034713.

Sakar MK, Petereit F, Nahrstedt A. 1993. Two phloroglucinol glucosides, flavan gallates and flavonol glycosides from *Sedum sediforme* flowers. Phytochemistry. 33:171–174.

Wen J, Qiao Y, Xiong Zl, Li FM. 2014. Simultaneous determination of seven constituents in Si-Ni-San decoction and a compatibility comparison study using HPLC-UV. Nat Prod Res. 28:1025–1029.

Wilzer KA, Fronczek FR, Urbatsch LE, Fischer NH. 1989. Coumarins from *Aster praealtus*. Phytochemistry. 28:1729–1735.

Yu SL, Ma L, Wu ZF, Zhao SX, Wang L, Ye WC, Zhang J, Yin ZQ. 2015. Non-C_{21} steroids from the rhizome of *Cynanchum stauntonii*. J China Pharm Univ. 46:426–430.

Zhang YY, Ye WC, Fan CL, Yin ZQ, Wang H, Zhao SX. 2005. A new coumarin glucoside from the fruits of *Citrus aurantium*. Chin J Nat Med. 3:141–143.

Zhang O, Wang HF, Zhang XL, Zhang WY, Pei YH, Chen G. 2015. Isolation and identification of chemical constituents from Aurantii Fructus Immaturus. J Shenyang Pharm Univ. 32:22–25.