Characterization of diterpenoids in the bark of *Pseudolarix kaempferi* by HPLC-ESI/MS^n

LIU Peng[1, 2], SUN Jiang-hao[1], XU Man[1], GUO Hui[1], GUO Hong-zhu[1, 3*], KANG Jie[1], HAN Jian[1], WANG Bao-rong[1], GUO De-an[1*]

(*1. Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University Health Science Center, Beijing 100083, China; 2. State Intellectual Property Office of The P.R.C., Beijing 100088, China; 3. Beijing Institute for Drug Control, Beijing 100035, China*)

Abstract: Fragmentation behavior of diterpenoids was investigated by ESI/MS^n and the qualitative analysis of diterpenoids in the bark of *Pseudolarix kaempferi* was performed using high-performance liquid chromatography/multi-stage mass spectrometry (HPLC-ESI/MS^n). The characteristic fragmentation behaviors of the diterpenoids are the cleavages of the lactone ring and C_4-O bond. Furthermore, the eliminations of substituent groups at C-18, C-7 and C-8 can also be observed in the MS^n (n = 3−4) spectra. For C-4 acetoxy subsititued diterpenoids, $[M+Na-60]^+$ and $[M-H-104]^-$ are the base peaks of MS^2 spectra in the positive and negative ionization modes, respectively. For C-4 hydroxyl subsititued diterpenoids, $[M+Na-44]^+$ and $[M-H-62]^-$ are the base peaks of MS^2 in the positive and negative ionization modes, respectively. For C-18 glucosylated or esterized diterpenoids, $[M+Na-44]^+$ is the base peak of MS^2 spectra in positive ionization mode. These fragmentation rules were successfully exploited in the identification of diterpenoids in methanol/water (6∶4) extract of *P. kaempferi* by LC-MS in positive ionization mode. A total of 9 diterpenoids were identified or tentatively characterized, and one of them is reported here for the first time. The described method could be utilized for the sensitive and rapid qualitative analysis of *P. kaempferi*.

Key words: *Pseudolarix kaempferi*; diterpenoid; quality control; qualitative analysis; HPLC-ESI/MS^n; fragmentation

CLC number: R917 **Document code**: A **Article ID**: 0513-4870 (2011) 02-0213-08

土荆皮中二萜类化合物的 HPLC-ESI/MS^n 鉴定

刘 鹏[1, 2], 孙江浩[1], 徐 曼[1], 郭 慧[1], 郭洪祝[1, 3*], 康 洁[1], 韩 健[1], 王宝荣[1], 果德安[1*]

(1. 北京大学医学部药学院天然药物学系, 北京 100083; 2. 国家知识产权局, 北京 100088; 3. 北京市药品检验所, 北京 100035)

摘要: 使用高效液相色谱-多级质谱法 (HPLC-ESI/MS^n) 研究了土荆皮二萜的 ESI/MS^n 裂解行为, 并对土荆皮中的二萜类化合物进行了定性分析。土荆皮二萜的特征裂解是内酯环和 C_4-O 键的断裂。此外, C-18、C-7 和 C-8 位取代基的离去在多级质谱 (MS^n, n = 3～4) 中也可观察到。对于 C-4 位乙酰氧基取代的二萜, $[M+Na-60]^+$ 和$[M-H-104]^-$分别是正、负离子模式下二级质谱的基峰。对于 C-4 位羟基取代的二萜, $[M+Na-44]^+$ 和$[M-H-62]^-$分别是正、负离子模式下二级质谱的基峰。对于 C-18 位葡萄糖苷化或酯化的二萜, $[M+Na-44]^+$ 是正离子模式下

Received 2010-11-20.
Project supported by the National High Technology Research and Development Program of China (Grant No. 2003AA2Z2030) and program for Changjiang Scholar and Innovative Team in University (Grant No. 985-2-063-112).
*Corresponding author Tel / Fax: 86-10-82805107, E-mail: guohz@bjmu.edu.cn;
Tel / Fax: 86-10-82802700, E-mail: gda@bjmu.edu.cn

二级质谱的基峰。利用上述裂解规律, 使用 LC-MS 方法在正离子模式下鉴定了土荆皮甲醇-水 (6∶4) 提取物中的二萜类化合物, 共有 9 个二萜被鉴定或初步推测, 其中一个是首次报道的化合物。所述方法可用于土荆皮的灵敏快速定性分析。

关键词: 土荆皮; 二萜; 质量控制; 定性分析; 高效液相色谱-多级质谱; 裂解

Tu-Jing-Pi, the root and trunk bark of *Pseudolarix kaempferi* Gord. (Pinaceae), is commonly used as a traditional Chinese medicine for the treatment of skin diseases caused by fungal infections. Pharmacological research revealed that it had significant antifungal[1], antitumor[2] and antifertility activities[3], as well as potent sclerosing effect on gallbladder in rabbit[4]. Since 1980s, systematic phytochemical studies on the bark, seeds and leaves of *P. kaempferi* have led to the isolation of more than seventy compounds, including diterpenoids[1, 2], triterpenoids[5], triterpene lactones[6] and phenolic compounds[7]. The characteristic diterpenoids obtained from the bark were reported to be responsible for the biological activities of this drug[1−3].

Therefore, the quality control of *P. kaempferi* should be focused on the analyses of the diterpenoids, which are of great importance for the quality of this drug. In our previous researches, a quantitative HPLC-DAD method has been established for simultaneous determination of seven major diterpenoids in *P. kaempfer*[8]. Furthermore, qualitative analysis of the drug is also necessary to ensure its authenticity and efficiency. However, HPLC-UV method is sometimes incompetent for the qualitative analysis because of the limitation of available reference standards necessary for the identification and the limited structural information provided by UV detector. High-performance liquid chromatography/multi-stage mass spectrometry (HPLC-MS^n) technique combines the high separation ability of HPLC and the structural characterization function of MS and can rapidly assign the peaks without reference standards. Moreover, the sensitive and selective MS detector allows detection and identification of minor or even trace constituents from a micro-scale sample. Therefore, it is becoming an ever-increasing popular tool in the rapid characterization of chemical constituents in plant extracts[9, 10].

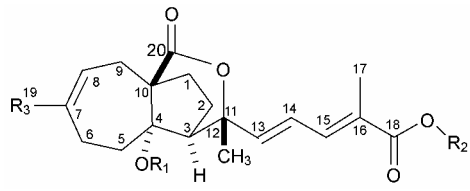
The fragmentation mechanisms of diterpenoids isolated from *P. kaempferi* in electron impact (EI) source have been reported in 1984[11]. Subsequently, no extensive investigation has been performed on their mass spectra. Electrospray ionization (ESI) is one of the most frequently used ion sources in HPLC-MS^n analysis. In this paper, the ESI/MS fragmentation behavior of diterpenoids was studied for the first time, and was further applied to the identification of diterpenoids in a methanol/water (6∶4) extract of *P. kaempferi*. Eight diterpenoids were identified, and one new component is tentatively characterized. The established method was valuable and dependable for the sensitive and rapid identification of diterpenoids in *P. kaempferi*.

Materials and methods

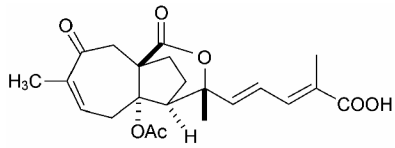
Chemicals and reagents Pseudolaric acid A, pseudolaric acid B, pseudolaric acid C, pseudolaric acid C_2, deacetylpseudolaric acid A, 11*S*-deacetylpseudolaric acid A, demethoxydeacetoxypseudolaric acid B, pseudolaric acid F, pseudolaric acid G, pseudolaric acid A *O-β-D*-glucopyranoside, pseudolaric acid B *O-β-D*-glucopyranoside, deacetylpseudolaric acid A *O-β-D*-glucopyranoside, deacetylpseudolaric acid A 2, 3-dihydroxypropyl ester, and deacetylpseudolaric acid B 2, 3-dihydroxypropyl ester (Figure 1) were isolated from the bark of *P. kaempferi* and identified by their NMR and MS data. Their purities were not less than 95% by HPLC analysis. The bark of *P. kaempferi* was identified by Dr. GUO Hongzhu and a voucher specimen (040309TJP01) was deposited in the Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University Health Science Center, China.

HPLC grade methanol, analytical grade acetic acid and other solvents used for compound isolation were all purchased from Beijing Chemical Engineering Factory (Beijing, China). The deionized water was prepared from Millipore water purification system (Millipore, Milford, MA, USA) and was filtered with 0.45 μm membranes.

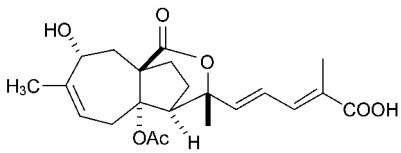
Sample preparation The dried powders of *P. kaempferi* samples (0.2 g, 60 mesh) were accurately weighed and soaked in 10 mL of methanol/water (6∶4) solution at room temperature for 0.5 h then extracted at 80 ℃ for 0.5 h. The resultant mixture was adjusted to the original weight and filtered through filter paper and then 0.45 μm membrane. Aliquots of 10 μL were injected for LC-MS analysis.



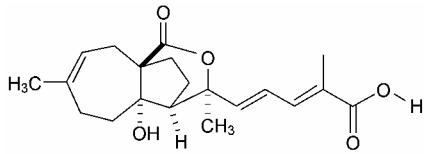
		R_1	R_2	R_3
1	Pseudolaric acid A (PA)	Ac	H	Me
2	Pseudolaric acid B (PB)	Ac	H	COOMe
3	Pseudolaric acid C_2 (PC_2)	Ac	H	COOH
6	Pseudolaric acid C (PC)	H	H	COOMe
7	Demethoxydeacetoxypseudolaric acid B (DDPB)	H	H	COOH
8	Deacetylpseudolaric acid A (DPA)	H	H	Me
10	Pseudolaric acid A *O-β-D* glucopyranoside (PAG)	Ac	Glc	Me
11	Pseudolaric acid B *O-β-D* glucopyranoside (PBG)	Ac	Glc	COOMe
12	Deacetylpseudolaric acid A *O-β-D*-glucopyranoside (DPAG)	H	Glc	
13	Deacetylpseudolaric acid A 2, 3-dihydroxypropyl ester (DPAP)	H	$CH_2CH(OH)CH_2OH$	Me
14	Deacetylpseudolaric acid B 2, 3-dihydroxypropyl ester (DPBP)	H	$CH_2CH(OH)CH_2OH$	COOMe



4 Pseudolaric acid F (PF)



5 Pseudolaric acid G (PG)



9 11*S*-Deacetylpseudolaric acid A (11*S*-DPA)

Figure 1 Structures of 14 diterpenoids

HPLC conditions The analyses were performed using an Agilent 1100 liquid chromatography system (Agilent, Waldbronn, Germany) equipped with a quaternary pump, a diode-array detector (DAD), an autosampler, and a column compartment. The samples were separated on an Inertsil ODS-3 column (250 mm × 4.6 mm, 5 μm). The mobile phase consisted of methanol and water containing 0.3% acetic acid. A gradient program was used as follows: a linear gradient from 55% methanol to 90% methanol in 40 min. The mobile phase flow rate was 0.6 mL·min^{-1}, and column temperature was set at 40 ℃. The DAD recorded UV spectra in the range from 190 − 400 nm, and the HPLC chromatogram was monitored at 262 nm.

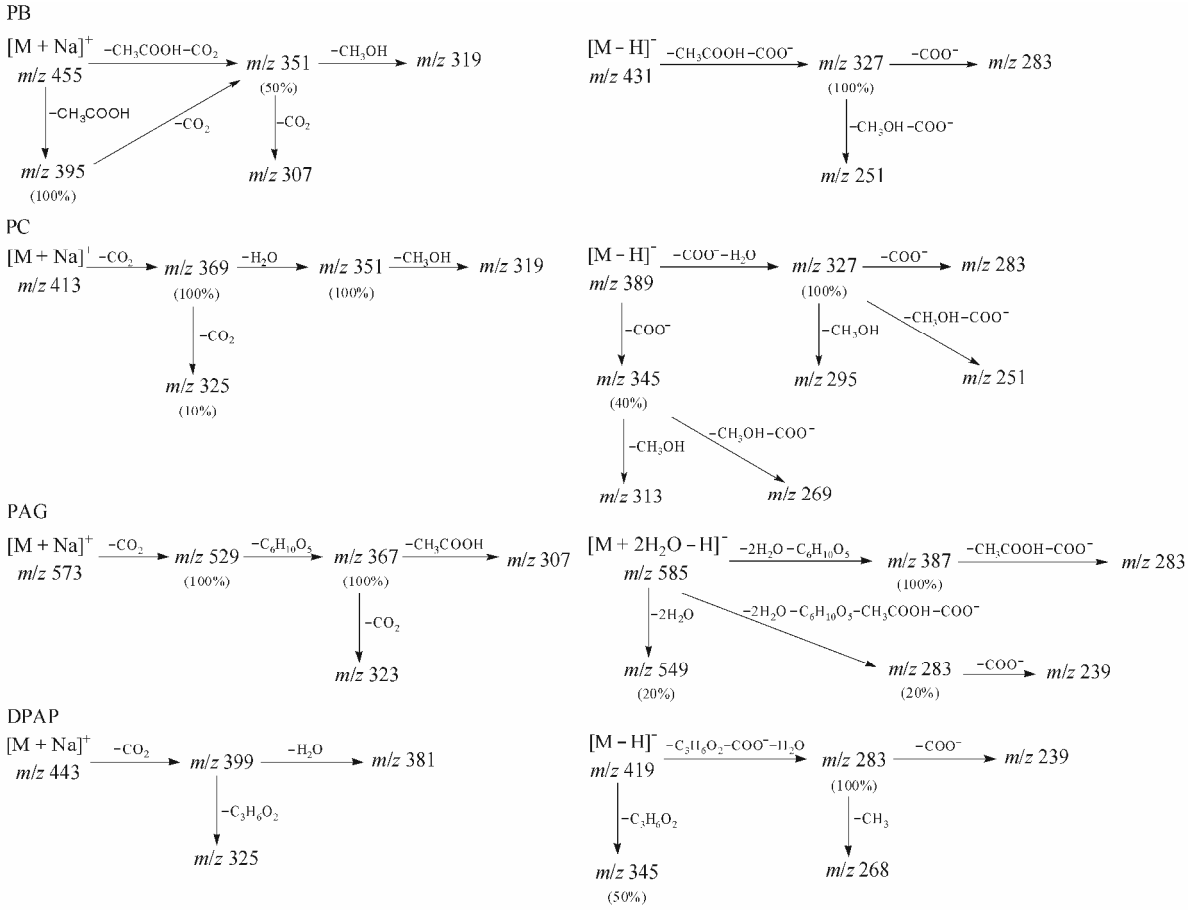
Mass spectrometry For HPLC/MS analysis, a Finnigan LCQ Advantage ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA) was connected to the Agilent 1100 HPLC system via an ESI interface. The LC effluent was introduced into the ESI source in a post-column splitting ratio of 3∶1. Ultra-high purity helium (He) was used as the collision gas and high purity nitrogen (N_2) as the nebulizing gas. Pure standards were dissolved in MeOH-H_2O (6∶4) to obtain solutions containing 0.1 mg·mL^{-1} and infused into the ESI source using a syringe pump at a flow rate of 2.5 μL·min^{-1}. The operating parameters were optimized for each standard. The operating parameters in the positive ionization mode for plant extracts were as follows: ion spray voltage, 4.5 kV; sheath gas (N_2) pressure, 50 arbitrary units; auxiliary gas (N_2) pressure, 10 units; capillary temperature, 300 ℃; capillary voltage, 30 V. For full-scan MS analysis, the spectra were recorded in the range of *m*/*z* 100 − 800. A data-dependent program was used in the liquid chromatography/tandem mass spectrometry analysis so that the two most abundant ions in each scan were selected and subjected to MS^n (n=2−4) analyses. The collision-induced dissociation (CID) energy was adjusted to 50%. The isolation width of the precursor ions was 1.5 Th.

Results and discussion

1 Tandem mass spectrometry of pure standards

The ionization of diterpenoids was performed in both positive and negative ionization modes and the responses were good in the both of the two modes. So the fragmentation behaviors of diterpenoids in the both of the two modes were investigated and compared.

The 14 reference compounds were introduced into the ESI source by continuous infusion. The base peak ions were subjected to MS^2 fragmentation and the prominent ions in each MS^2 spectrum were then selected for further MS^n analysis (n = 3 – 4). Normalization collision energy was in the range of 20%−50%. Except for the lactone ring, the skeleton of the diterpenoids is stable in the ESI source. Hence the substitution groups determine the fragmentation behaviors of the diterpenoids. The fragmentation patterns of the diterpenoids could be classified into four groups according to their substitutions at C-4 and C-18 positions. The fragmentation pathways are shown in Scheme 1.



Scheme 1 Major fragmentation pathways of diterpenoids in positive and negative ionization modes

1.1 Group Ⅰ: C-4 acetoxy and C-18 carboxyl

Compounds in this group have acetoxy and carboxyl substituted at C-4 and C-18, respectively, including PA, PB, PC_2, PF and PG. In positive ionization mode, the full scan mass spectrum of PB gave a $[M+Na]^+$ ion at *m*/*z* 455 and this ion was selected for MS^n (n = 2 – 4) analysis. A base peak ion at *m*/*z* 395 ($[M+Na-60]^+$) was observed in the MS^2 spectrum, resulting from the loss of CH_3COOH. The ions at *m*/*z* 411 ($[M+Na-44]^+$) and *m*/*z* 351 ($[M+Na-104]^+$) were attributed to elimination of CO_2 and concurrent losses of CH_3COOH and CO_2, respectively. The ion at *m*/*z* 395 was subjected to MS^3 analysis and produced a $[M+Na-60-44]^+$ ion at *m*/*z* 351, which underwent loss of CO_2 and CH_3OH to generate ions at *m*/*z* 307 and *m*/*z* 319 in MS^4 spectrum, respectively. The loss of 32 Da (CH_3OH) provided characteristic fragmentation information for compounds with C-7 methyl formate substitution. The results obtained in this study led to a proposed fragmentation pathway of PB in positive ionization mode as illustrated in Figure 2. PB could lose two molecules of CO_2, owing to its structural moieties of lactone ring and C-18 carboxyl group. It was assumed that PB underwent previous loss of lactone ring and following loss of C-18 carboxyl because the cleavage of lactone ring generated an ion with very stable conjugated structure, i.e. the ion at *m*/*z* 351 in Figure 2.

The other compounds in this group, viz. PA, PC_2, PF and PG, also showed prominent ions of $[M+Na-60]^+$, as well as the minor ions of $[M+Na-104]^+$ in their MS^2 spectra. The product ions could further lost CO_2 and the substituent groups at C-8, showing characteristic fragmentation information corresponding to their structures. For example, the loss of C-8 carbonyl and hydroxyl group resulted in the ions of *m*/*z* 293 (425→321→293) and *m*/*z* 305 (427→323→305) in MS^3 of PF and PG, respectively.

In negative ionization mode, compounds in this group usually gave $[M-H]^-$ ions, which were subjected to further fragmentation and yielded $[M-H-104]^-$ ions as base peaks, as well as the $[M-H-60]^-$ ions. The subsequent losses of COO^- could also be observed in MS^3 spectra of $[M-H-104]^-$ ions. Moreover, the loss of the C-7 substituent group (methyl free radical) was

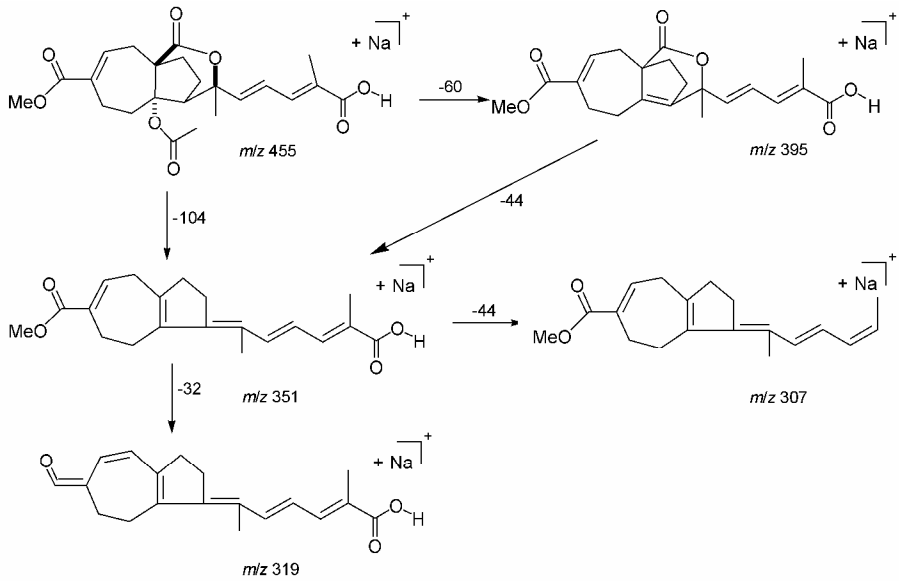


Figure 2 The proposed fragmentation pathway of PB in positive ionization mode

not found in positive ionization mode.

1.2 Group Ⅱ: C-4 hydroxyl and C-18 carboxyl Compounds in this group contain C-4 hydroxyl and C-18 carboxyl, including PC, DDPB, DPA and 11*S*-DPA. In positive ionization mode, fragmentation of the $[M+Na]^+$ ion of PC at *m*/*z* 413 involved initial loss of CO_2 to generate the $[M+Na-44]^+$ ion (*m*/*z* 369). The ion at *m*/*z* 369 was then subjected to MS^3 analysis and produced a prominent ion ($[M+Na-44-18]^+$) at *m*/*z* 351, which then yielded an ion at *m*/*z* 319 in the MS^4 spectrum, through loss of a molecule of CH_3OH. Another minor ion at *m*/*z* 325 was also observed in the MS^3 spectrum of the ion at *m*/*z* 369, resulting from the loss of CO_2.

DDPB had almost the same fragmentation pathway as PC, except for the loss of CH_3OH. DPA and 11*S*-DPA are configuration isomers and their fragmentation behaviors are identical. Fragmentation of $[M+Na]^+$ ion at *m*/*z* 369 was triggered by initial loss of CO_2 (369→325), followed by simultaneous loss of CO_2 and CH_3 (325→266).

In negative ionization mode, compounds in this group usually showed $[M-H]^-$ ions, which were selected for further MS^2 analyses and generated $[M-H-62]^-$ ions as base peaks, as well as $[M-H-44]^-$ ions. The $[M-H-62]^-$ ions were originated from concurrent loss of COO^- and H_2O from $[M-H]^-$ ion, and the subsequent losses of COO^-, CH_3OH or CH_3 could also be observed in MS^3 spectra corresponding to their substitutions at C-18 and C-7.

1.3 Group Ⅲ: C-18 carboxylic glucoside The C-18 carboxyl of this group of compound is glucosylated, such as PAG, PBG and DPAG. In positive ionization mode, the $[M+Na]^+$ ion at *m*/*z* 573 of PAG underwent successive loss of 44 Da (CO_2) and then 162 Da (glucosyl). The *m*/*z* 367 ion obtained subsequently produced ions at *m*/*z* 323 and *m*/*z* 307 via the same pathways as PA. PBG and DPAG had almost the same fragmentation pattern as PAG.

In negative ionization mode, compounds in this group usually showed remarkable $[M+2H_2O-H]^-$ ions, which were selected for further MS^2 analyses and ions formed via the loss of 198 Da ($[M+2H_2O-H-36-162]^-$) were observed as base peaks and their further fragmentation behaviors were the same as the corresponding aglycones.

1.4 Group Ⅳ: C-18 carboxylic ester This group of compounds has a 2, 3-dihydroxypropyl group attached to C-18, forming an ester of the acid, such as DPAP and DPBP. The MS^2 spectrum of DPAP from the $[M+Na]^+$ ion at *m*/*z* 443 in positive ionization mode gave a $[M+Na-44]^+$ ion at *m*/*z* 399. The MS^3 spectrum of *m*/*z* 399 yielded *m*/*z* 381 and *m*/*z* 325, corresponding to loss of a molecule of H_2O and a 2, 3-dihydroxypropyl group, respectively. DPBP exhibited the same fragmentation pathway as DPAP.

In negative ionization mode, the $[M-H]^-$ ions of DPAP and DPBP were observed. $[M-H-74]^-$ and $[M-H-136]^-$ ions in the MS^2 spectra were observed owing to elimination of 2, 3-dihydroxypropyl group and concurrent losses of COO^-, H_2O and 2, 3-dihydroxypropyl group, respectively.

Except for the lactone ring, the skeleton of the diterpenoids is stable in the ESI source in positive and

negative ionization modes, therefore the substitution groups determine the fragmentation behaviors of the diterpenoids. The typical fragmentation behaviors of the diterpenoids are the cleavages of the lactone ring and C_4-O bond. Furthermore, the eliminations of substituent groups at C-18, C-7 and C-8 can also be observed in the MS^3 and MS^4 spectra. The major difference of the fragmentation behaviors between these two ionization modes is the base peak in the MS^2 spectrum. In positive ionization mode, $[M+Na-60]^+$ or $[M+Na-44]^+$ ion is the base peak in the MS^2 spectrum, which is attributed to the loss of C-4 acetoxy or laction ring. While $[M-H-104]^-$ or $[M-H-62]^-$ ion is the base peak in negative ionization mode, corresponding to the concurrent loss of lactone ring and C-4 substitution, i.e. acetoxy or hydroxyl.

2 HPLC-ESI/MS^n analysis of *P. kaempferi* extract

Positive ionization mode was used in the HPLC/ESI-MS^n analysis of *P. kaempferi* extract because the acetic acid in the mobile phase strongly inhibited the ionization of diterpenoids in negative ionization mode. The HPLC-UV and total ion current (TIC) profiles of the extract of *P. kaempferi* crude drug were shown in Figure 3. Nine diterpenoids were detected and characterized from the extract of *P. kaempferi* (Table 1). Eight of them were unambiguously identified by comparing their retention times, UV and mass spectra with reference standards. For the unknown peak, the structure was tentatively established based on its MS^n analysis, according to the general fragmentation rules summarized above as well as the biogenesis.

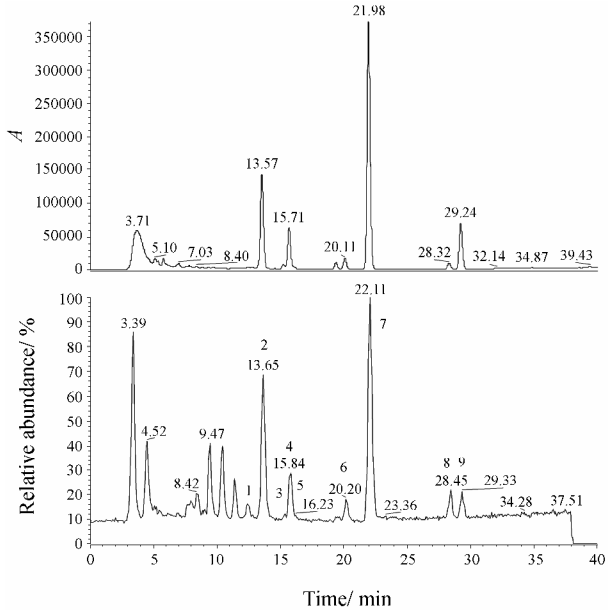


Figure 3 The HPLC-UV chromatogram monitored at 262 nm and ESIMS total ion current profile of *P. kaempferi*

2.1 Group Ⅰ Peak 8 (retention time (t_R) = 28.53 min) gave $[M+Na]^+$ ions at *m*/*z* 469 and yielded product ion at *m*/*z* 409 as base peak, as well as minor ions at *m*/*z* 425 and 365 in MS^2 analysis. The ion at *m*/*z* 409 was assigned as the loss of CH_3COOH from the precursor ion at *m*/*z* 469, indicating the presence of C-4 acetoxy, which further generated ion at *m*/*z* 365 owing to the cleavage of lactone ring. The ion at *m*/*z* 377 was observed in MS^3 spectrum of *m*/*z* 409 and was attributed

Table 1 Characterization of diterpenoids in *Pseudolarix kaempferi* by HPLC-ESI/MS^n (*m*/*z*, % of relative abundance). *New compound

No.	t_R/min	Assiged identity	UV λ_{max}/nm	$[M+Na]^+$ *m*/*z*	HPLC-ESI/MS^n *m*/*z* (% base peak)
1	12.87	DPBP	236	487	MS^2 [487]: 443 (100), 425 (10)
2	13.54	PBG	266	617	MS^2 [617]: 573 (60), 557 (35), 513 (10), 455 (100), 395 (35)
					MS^3 [617→455]: 413 (15), 395 (100), 351 (15)
					MS^4 [617→455→395]: 363 (100), 351 (65)
3	15.48	PC_2	262	441	MS^2 [441]: 397 (5), 381 (100), 337 (70)
					MS^3 [441→381]: 337 (100)
4	15.75	PC	262	413	MS^2 [413]: 369 (100), 351 (1)
					MS^3 [413→369]: 351 (100), 325 (25)
5	16.15	DPAP	242	443	MS^2 [443]: 381 (100), 327 (25)
6	20.08	PAG	266	573	MS^2 [573]: 529 (100), 367 (10)
					MS^3 [573→529]: 367 (100)
					MS^4 [573→529→367]: 307 (100)
7	22.02	PB	260	455	MS^2 [455]: 411 (20), 395 (100), 351 (60)
					MS^3 [455→395]: 377 (20), 363 (50), 351 (100)
8*	28.53	PI	252	469	MS^2 [469]: 425 (20), 409 (100), 365 (50)
					MS^3 [469→409]: 391 (5), 377 (15), 365 (100)
9	29.25	PA	260	411	MS^2 [411]: 367 (90), 351 (100), 307 (90)

to the emanation of CH_3OH, suggesting the existence of C-7 methyl formate. The molecular mass of Peak 8 was 44 Da higher than known compound PF, in conjunction with the C-7 methyl formate and biogenesis relationship, it was therefore tentatively characterized as a new compound pseudolaric acid I (PI) as showed in Figure 4.

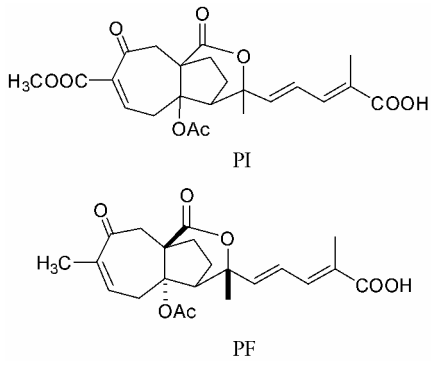


Figure 4 Proposed structure of PI

2.2 Group Ⅱ Peak 4 (t_R = 15.75 min) gave $[M+Na]^+$ ions at *m*/*z* 413 and produced ion at *m*/*z* 369 as base peak, as well as minor ion at *m*/*z* 351 in MS^2 analysis. The ion at *m*/*z* 351 was assigned as the concurrent loss of CO_2 and H_2O from the precursor ion at *m*/*z* 413, indicating the presence of C-4 hydroxyl. Hence, Peak 4 was identified as pseudolaric acid C (PC) by comparing with the reference standard.

2.3 Group Ⅲ Peak 2 (t_R = 13.54 min) gave $[M+Na]^+$ ions at *m*/*z* 617 and its MS^2 spectra produced ions at *m*/*z* 573, 557 and 455, corresponding to losses of CO_2, CH_3COOH and glycosyl, respectively. The ion at *m*/*z* 455 was further subjected to MS^3 analysis and yielded ion at *m*/*z* 395 as base peak, indicating the presence of C-4 acetoxy. The ion at *m*/*z* 413 in MS^3 was assigned as the loss of CH_3OH from *m*/*z* 455, suggesting the existence of C-7 methyl formate. The MS^4 spectrum of *m*/*z* 395 gave ions at *m*/*z* 363 and 351, which were attributed to the cleavages of CH_3OH and CO_2, respectively. Thus, Peak 2 was identified as pseudolaric acid B *O-β-D* glucopyranoside (PBG) by comparing with the reference standard.

2.4 Group Ⅳ Peak 1 (t_R = 12.87 min) gave $[M+Na]^+$ ions at *m*/*z* 487 and its MS^2 spectra produced ions at *m*/*z* 443 and 425, corresponding to loss of CO_2 and concurrent losses of CO_2 and H_2O, respectively. Therefore, Peak 1 was identified as deacetylpseudolaric acid B 2, 3-dihydroxypropyl ester (DPBP) by comparing with the reference standard. The identification of DPAP and DPBP from the methanol/water extract of *P. kaempferi* unambiguously demonstrated that they are natural products originated from the plant, but not artefacts.

Conclusions

In this study, the fragmentation behaviors of diterpenoids in the bark of *P. kaempferi* were investigated by using HPLC-ESI/MS^n method. Except for the lactone ring, the skeleton of the diterpenoids is stable in the ESI source in the two ionization modes. The typical fragmentation behaviors of the diterpenoids are the cleavages of the lactone ring and C_4-O bond. Furthermore, the eliminations of substituent groups at C-18, C-7 and C-8 can also be observed in the MS^n (n = 3−4) spectra. The major difference of the fragmentation behaviors between these two ionization modes is the base peak in the MS^2 spectrum. For C-4 acetoxy substituted diterpenoids, $[M+Na-60]^+$ and $[M-H-104]^-$ are the base peaks in the positive and negative ionization mode, respectively. For C-4 hydroxyl substituted diterpenoids, $[M+Na-44]^+$ and $[M-H-62]^-$ are the base peaks in the positive and negative ionization mode, respectively. For C-18 glucosylated or esterized diterpenoids, $[M+Na-44]^+$ is the base peak in positive ionization mode.

These deduced rules were successfully exploited in the identification of diterpenoids in the bark of *P. kaempferi* by LC/MS in positive ionization mode. A total of 9 diterpenoids were identified or tentatively characterized, and one of them is reported here for the first time. The described method could be utilized for the sensitive and rapid qualitative analysis of *P. kaempferi* and its pharmaceutical preparations. This method is of great significance for the characterization of minor diterpenoids *in vivo* after dosing with *P. kaempferi*, so as to clarify their metabolic pathways and remedial mechanisms.

References

[1] Yang SP, Dong L, Wang Y, et al. Antifungal diterpenoids of *Pseudolarix kaempferi* and their structure-activity relationship study [J]. Bioorg Med Chem, 2003, 11: 4577−4584.

[2] Liu P, Guo HZ, Wang WX, et al. Cytotoxic diterpenoids from the bark of *Pseudolarix kaempferi* and their structure-activity relationships [J]. J Nat Prod, 2007, 70: 533−537.

[3] Wang WC, Lu RF, Zhao SX, et al. Comparison of early

pregnancy-terminating effect and toxicity between pseudolaric acid A and B [J]. Acta Pharmacol Sin, 1988, 9: 445−448.

[4] Li H, Xu ZS. Sclerosing effect of tincture of cortex pseudolsricis compound on gallbladder in rabbits: an experimental research [J]. J Nanjing Rail Med Col (南京铁道医学院学报), 1995, 14: 74−76.

[5] Chen K, Li ZL, Pan DJ. Studies on the triterpenic constituents of Tu Jing Pi [J]. Acta Chim Sin (化学学报), 1990; 48: 591−595.

[6] Chen GF, Li ZL, Pan DJ. The isolation and structural elucidation of four novel triterpene lactones, pseudolarolides A, B, C, and D, from *Pseudolarix kaempferi* [J]. J Nat Prod, 1993, 56: 1114−1122.

[7] Yang SP, Wang Y, Wu Y, et al. Chemical constituents from *Pseudolarix kaempferi* [J]. Nat Prod Res, 2004, 18: 439−446.

[8] Liu P, Guo H, Guo HZ, et al. Simultaneous determination of seven major diterpenoids in *Pseudolarix kaempferi* by high-performance liquid chromatography DAD method [J]. J Pharm Biomed Anal, 2007, 44: 730−736.

[9] Ye M, Guo DA. Investigations of taxoids by electrospray and atmospheric pressure chemical ionization with tandem mass spectrometry [J]. Rapid Commun Mass Spectrom, 2005, 19: 818−824.

[10] Ye M, Guo DA, Ye G, et al. Analysis of homoisoflavonoids in *Ophiopogon japonicus* by HPLC-DAD-ESI-MS^n [J]. J Am Soc Mass Spectrom, 2005, 16: 234−243.

[11] Huang ZH, Liu BN, Ying BP, et al. Studies on the mass spectra of pseudolaric acids and their derivatives [J]. Acta Chim Sin (化学学报), 1984, 42: 886−892.

· 消 息 ·

STN®成为中国化学及药物研发行业的优选工具

当中国乃至全球企业纷纷将目光投向专利保护时, 专利信息检索与知识产权保护显得尤为重要。与昂贵的研发费用以及麻烦的侵权纠纷相比较, 越来越多的企业已将专利检索视为其通往成功决策的一条捷径。美国化学文摘社 (CAS) 联合德国莱布尼茨学会卡尔斯鲁厄专业信息中心 (FIZ Karlsruhe) 及日本化学情报协会 (JAICI) 共同运营 STN, STN 所提供的 250 多个数据库涵盖的学科包括生物科学、生物技术、商业、化学、能源与环境、工程学、健康与安全、材料学、医学以及纳米科技等等。同时, STN 能提供 CAplus℠、INPADOCDB 和德温特世界专利索引 (DWPI) 数据库的平台, 让研发人员可以一次性确定可专利性、已有新技术、侵权以及专利法律状态等信息, 节约宝贵的研发时间。

STN 不仅仅只是庞大数据库的集合, 同时还结合了先进的搜索工具来实现高效而精准的信息检索。STN Express®、STN on the Web℠、STN Easy™ 是三个不同功能的搜索界面, 通过它们均能访问 STN 数据库。其中 STN on the Web 可以同时检索多个数据库, 为用户提供使用词典来查找和选择多个数据库的相关术语, 节省时间的同时增加了检索广泛度。用户也可根据自己的需求来选择 STN Express 或 STN Easy 界面, 进行访问 STN 数据库, 检索更具人性化且高效便捷。STN AnaVist™ 与 STN Viewer™ 则是 STN 实现可分析、可视化及专利评估的工具。其中 STN AnaVist 软件可配合 STN Express 或 STN on the Web 将分析和视像化性能运用于答案集中, 并且可查看文件中 13 种不同内容之间的关系, 如公司、发明者、年份等。集全面数据库与强大功能于一身的 STN, 现已成为众多中国企业以及科研机构进行专利信息检索的优选工具。STN 中的数据库由科学家们进行维护与编辑, 每天或每周都会有数据库的更新。值得一提的是 STN 特有的 Marpat 数据库, 它所提供的 Markush 结构可以帮助企业确保扩大专利保护范围, 并在所有相似结构范围里隐藏真正有效的化合物, 从而使竞争者无法正确了解到企业研发的真正具有疗效的化合物。