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Short communication

Isolation and characterization of process-related impurities and degradation products in larotaxel

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ABSTRACT

The isolation and characterization of the process related impurities and degradation products of larotaxel drug substance were described. Forced degradation of larotaxel was carried out under acidic, basic, oxidation, light and thermal conditions to assess the nature of the impurities. The pure impurities were obtained by semi-preparative LC isolation and analyzed by NMR and MS. The structures of impurities were confirmed as 7,8-cyclopropyl baccatin III, 10-deacetyl larotaxel, 10-deacetyl-7, 8-cyclopropyl baccatin III, 7-acetyl-8-methyl larotaxel and 2′,13-bissidechain larotaxel.

1. Introduction

Larotaxel is a novel semisynthetic taxoid compound that has the similar active mechanism to docetaxel. The in vitro studies showed that larotaxel acted well in the cell lines which were resistant to paclitaxel and docetaxel [1]. Additionally, larotaxel had the ability to penetrate the blood–brain barrier, which may be a consequence of its decreased recognition by P glycoprotein (P-gp) [2]. The results of Phase II multicenter study demonstrated that larotaxel had good activity in patients with metastatic breast cancer (MBC) who had previously received taxane-based therapy with a higher overall response rate (ORR) (42%) in the nonresistant patient cohort than in the resistant cohort (19%) [3].

Larotaxel was prepared from 10-deacetyl baccatin III extracted from the needles of yew trees. Larotaxel is not yet official in any of the pharmacopoeia and no impurity study has been reported. According to International Conference on Harmonization (ICH) guideline on impurities in new drug substance, impurities at or above 0.1% should be identified for drugs with a maximum daily dose equal to or lesser than 2 g [4]. The present study describes the isolation and characterization of the process related impurities and degradation products of larotaxel drug substance. The pure impurities were isolated by semi-preparative LC and characterized using NMR and MS spectral data.

2. Experimental

2.1. Reagents and samples

Larotaxel (>98% pure) was synthesized in Shandong Target Drug Research Co. Ltd. (Yantai, China). The scheme for synthesis of larotaxel shown in Fig. 1. HPLC grade acetonitrile was purchased from Fisher Scientific (NJ, USA). $CDCl_3$ was from Sigma–Aldrich Co. (St. Louis, MO, USA). Other chemicals were analytical grade. Milli-Q water was used throughout the study.

2.2. Analytical LC condition

The chromatographic separation was performed on Agilent 1200 HPLC system (Santa Clara, CA, USA) with UV detector. The HPLC method was developed for the analysis of the process related impurities and degradation products of larotaxel. Separations were achieved on DIKMA Diamonsil C18 column (Beijing, China) with 250 mm × 4.6 mm i.d., 5 μm particle size maintained at 30 °C. The mobile phase consisted of water (A) and acetonitrile (B) with a flow rate of 1 mL/min. The impurities were detected at 230 nm and eluted according to the step gradient by changing the percentage

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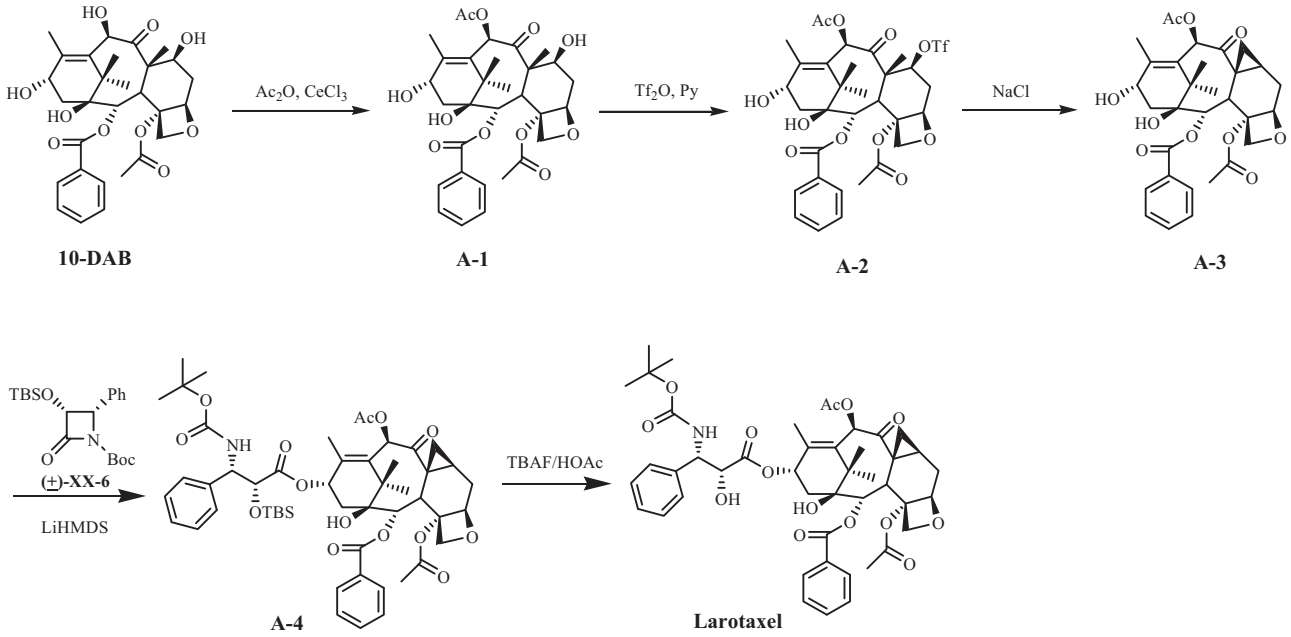


Fig. 1. Scheme for the synthesis of larotaxel.

of solvent B at different times, *T* (min)/% Solvent B = 0/55, 30/55, 60/90, 61/55, 70/55.

2.3. Forced degradation of larotaxel

Forced degradation was carried out under (1) 60 °C for 10 days (2) 0.1 N HCl solution for 4 h (3) 0.1 N NaOH solution for 2 h (4) 30% hydrogen peroxide solution for 4 h and (5) strong light (4500 lx) for 10 days. Both solid sample and solution of larotaxel were used in thermal and strong light degradation tests. Under thermal, oxidation and strong light conditions, no change in the sample purity was observed. However in acidic and basic conditions, three same degraded products (Impurities I, II and III) formed (Fig. 2).

2.4. Stability study

The stability study was performed with larotaxel drug substance kept in aluminum foil composite film and stored in climatic chamber at 25 ± 2 °C/60% ± 5% RH for 12 months. The stability samples were analyzed by HPLC method as described in Section 2.2. At the twelfth month, four degradations (Impurity I, II III and IV were observed in the stability samples.

2.5. Validation of analytical LC method

The specificity of HPLC method described in Section 2.2 was validated. The homogeneity of larotaxel peak in each forced degradation and stability samples was examined by peak purity testing utilizing DAD detector. The purity factor obtained from larotaxel peak was higher than threshold, which demonstrated the spectral homogeneity. The degradation products in forced degradation and stability samples were all separated from each other and from larotaxel. The detection limit and quantitation limit of larotaxel were 0.0054% (w/w) and 0.019% (w/w), which was determined by signal to noise (S/N) ratio method. All these proved that the method was specific and sensitive to the determination of impurities in larotaxel.

2.6. Isolation of impurities

The process related impurities and degradation products were isolated from base stressed and stability samples by semi-preparative HPLC. The semi-preparative HPLC system consisted of a Shimadzu LC-8A pump connected to Shim-pack PRO-ODS (250 mm × 20 mm; 15 μm, Shimadzu, Kyoto, Japan), a FRC-10A fraction collection trap and a SPD-10A detector (Kyoto, Japan). For the isolation of Impurity I, II and III, about 1.0 g larotaxel was used in the base degradation. Acetonitrile–water (50:50, v/v) was used as mobile phase with a flow rate of 10 mL/min. The wavelength of detection was 230 nm and the injection volume was 1 mL. Impurities I and II were isolated from stability sample using the mobile phase of acetonitrile–water (60:40, v/v) with a flow rate of 10 mL/min. The isolated fractions were respectively pooled together and concentrated on a rotaevaporator under vacuum. Five pure impurities about 30–50 mg were obtained with the purity over 95%. The purity of impurities was analyzed by HPLC method described in Section 2.2.

2.7. Mass spectrometry

Electrospray ionization and tandem mass spectrometry experiments were performed using a triple quadrupole mass spectrometer from Thermo-Fisher Scientific Inc. (Waltham, MA, USA). The positive ion electrospray data were obtained by switching the capillary voltage between +4000 V and −4000 V. Collision potential (30 V) and argen gas were used in the collision cell for MS–MS studies.

2.8. NMR spectroscopy

To characterize the structure of larotaxel and impurities, ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H–^{1}H COSY, HSQC, and HMBC spectrum were applied. The NMR spectroscopy was recorded on Bruker 400 MHz nuclear magnetic resonance spectrometer, using $CDCl_3$ as solvent and TMS as internal standard. The sample concentration was approximately 20 mg/mL.

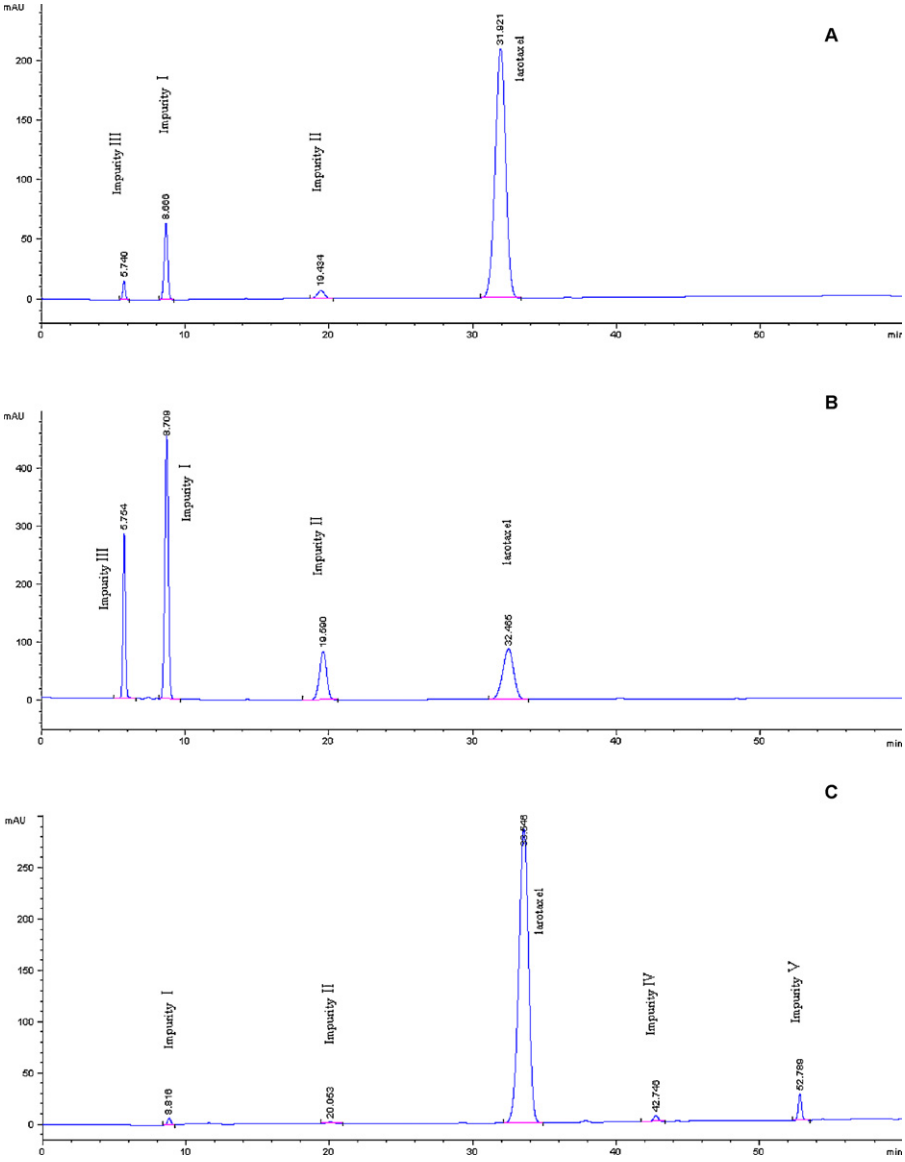


Fig. 2. Typical HPLC chromatogram of (A) acid stressed degradation (B) base stressed degradation (C) 12-month stability samples of larotaxel.

3. Results and discussion

3.1. Detection of impurities I–V

The typical HPLC chromatogram (Fig. 2.) of larotaxel and impurities was recorded using the LC method described in Section 2.2. The structure, relative retention time, molecular weight and nature of larotaxel and impurities were shown in Table 1. The spectral data were compared with those of larotaxel to characterize the structure of impurities.

3.2. Structure elucidation of impurities

3.2.1. Larotaxel

The ESI mass spectrum of larotaxel showed a sodium adduct $[M+Na]^+$ at m/z 854 and a protonated molecular ion $[M+H]^+$ at m/z 832. The fragmentation pathway of the protonated molecular ion at m/z 832 was obtained: m/z at 552, 492, 281, 225 and 181. The structure of larotaxel was confirmed by using ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H–^{1}H COSY, HSQC, and HMBC spectrum, and all NMR signals were assigned in Table 2. The ^{1}H NMR data were coincident with those reported [5].

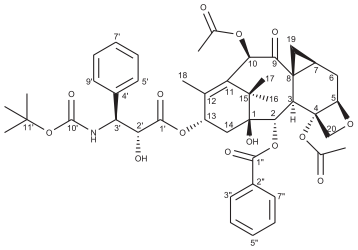
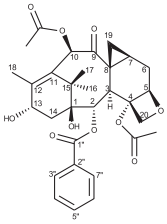
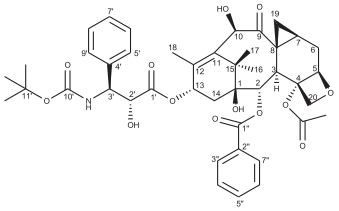
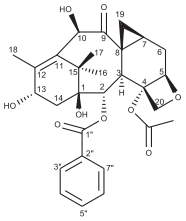
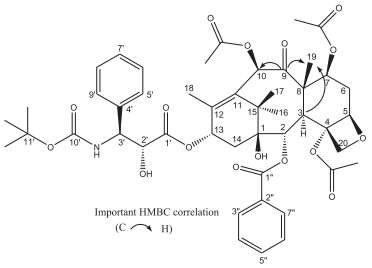
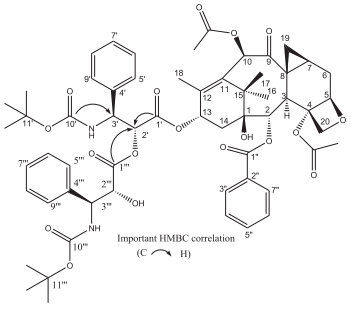
3.2.2. Impurity I

The ESI mass spectrum of Impurity I showed a sodium adduct $[M+Na]^+$ at m/z 591 and a protonated molecular ion $[M+H]^+$ at m/z 569, indicating that Impurity I had a molecular mass less than that of larotaxel by 263 Da. In ^{1}H and ^{13}C NMR spectrum, all side chain signals of larotaxel were absent. The retention time of Impurity I in HPLC chromatogram was same as the synthetic intermediate (A-3) of larotaxel. Thus Impurity I was confirmed as 7,8-cyclopropyl baccatin III. It is a new compound which was unreported.

3.2.3. Impurity II

The ESI mass spectrum of Impurity II gave a sodium adduct $[M+Na]^+$ at m/z 812 and a protonated molecular ion $[M+H]^+$ at m/z 790, indicating that Impurity II had a molecular mass less than that of larotaxel by 42 Da. In ^{1}H and ^{13}C NMR spectrum, the C10 acetyl proton signal and carbonyl signal were absent, which was

Table 1
Name, relative retention time (RRT), structure, molecular weight and nature of larotaxel and impurities.

Name	RRT (min)	Structure	Molecular weight	Nature
Larotaxel	1.00		831	Drug substance
7,8-Cyclopropyl baccatin III (Impurity I)	0.27		568	Process related acid and base stressed degradation stability
10-Deacetyl larotaxel (Impurity II)	0.61		789	Process related acid and base stressed degradation stability
10-Deacetyl-7, 8-cyclopropyl baccatin III (Impurity III)	0.18		526	Acid and base stressed degradation
7-Acetyl-8-methyl larotaxel (Impurity IV)	1.27		891	Process related stability
2′,13-Bissidechain larotaxel (Impurity V)	1.57		1094	Process related stability

present in larotaxel at 2.21 ppm and 169.6 ppm, respectively. The significant change was found in the ^{1}H chemical shift value of the C10 proton. Therefore, Impurity II was confirmed as 10-deacetyl larotaxel.

3.2.4. Impurity III

The ESI mass spectrum of Impurity III gave a sodium adduct $[M+Na]^{+}$ at m/z 549 and a protonated molecular ion $[M+H]^{+}$ at m/z 527, indicating that Impurity III had a molecular mass less than

Table 2
^{1}H and ^{13}C NMR assignments for larotaxel and impurities.

	Larotaxel		Impurity II		Impurity I		Impurity III		Imputity IV		Imputity V	
Position	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	79.4	–	79.4	–	79.4	–	79.5	–	78.6	–	79.5	–
2	80.0	5.66 (1H, d, J = 7.7 Hz)	80.1	5.66 (1H, d, J = 7.3 Hz)	80.0	5.65 (1H, d, J = 7.7 Hz)	80.1	5.63 (1H, d, J = 7.7 Hz)	74.4	5.67 (1H, d, J = 6.8 Hz)	80.1	5.66 (1H, d, J = 7.7 Hz)
3	38.5	4.08 (1H, d, J = 7.7 Hz)	38.3	4.13 (1H, d, J = 7.3 Hz)	38.5	4.25 (1H, d, J = 7.3 Hz)	38.6	4.23 (1H, d, J = 7.3 Hz)	47.1	3.92 (1H, d, J = 6.8 Hz)	38.6	4.12 (1H, d, J = 7.7 Hz)
4	79.6	–	79.5	–	79.3	–	79.2	–	81.1	–	79.6	–
5	84.8	4.72 (1H, d, J = 3.3 Hz)	84.7	4.73 (1H, d, J = 2.7 Hz)	84.7	4.73 (1H, d, J = 2.8 Hz)	84.8	4.75 (1H, d, J = 2.8 Hz)	83.9	4.94 (1H, d, J = 9.0 Hz)	84.8	4.77(1H, d, J = 3.3 Hz)
6	26.0	2.46(1H,dt, J = 16.1,4.4 Hz) 2.10(1H, d, J = 16.1 Hz)	26.0	2.43(1H,dt, J = 16.1,4.4 Hz) 2.13(1H,d, J = 16.1 Hz)	26.1	2.43(1H,dt, J = 15.4,4.4 Hz) 2.13(1H, d, J = 15.4 Hz)	26.0	2.45(1H,dt, J = 15.4,4.4 Hz) 2.12(1H,d, J = 15.4 Hz)	33.4	2.60 (1H, m), 1.82 (1H, m)	26.0	2.49(1H,dt, J = 16.1,4.4 Hz) 2.12(1H, d, J = 16.1 Hz)
7	32.0	1.37 (1H, m)	32.9	1.42 (1H, m)	32.5	1.37 (1H, m)	32.6	1.39 (1H, m)	71.4	5.56 (1H, dd, J = 10.2, 7.3 Hz)	32.1	1.38 (1H,m)
8	35.1	–	34.7	–	354.8	–	354.9	–	56.1	–	35.1	–
9	201.8	–	209.7	–	210.2	–	210.1	–	201.9	–	201.9	–
10	75.7	6.33 (1H, s)	75.5	5.01 (1H, s)	76.1	6.35 (1H, s)	76.0	5.05 (1H, s)	75.3	6.25 (1H, s)	75.7	6.36 (1H, s)
11	134.0	–	136.3	–	135.2	–	135.2	–	132.8	–	133.7	–
12	140.4	–	138.6	–	142.5	–	142.4	–	140.7	–	140.9	–
13	72.1	6.27 (1H, t, J = 8.4 Hz)	72.3	6.29(1H, t, J = 8.0 Hz)	67.6	4.85 (1H, t, J = 7.7 Hz)	67.7	4.86 (1H, t, J = 7.7 Hz)	72.3	6.18 (1H, t, J = 8.5 Hz)	71.1	6.43 (1H, t, J = 8.4 Hz)
14	35.8	2.38 (1H, m) 2.23 (1H, m)	36.1	2.35 (1H, m) 2.21 (1H, m)	38.8	2.35 (2H, m)	38.9	2.34 (2H, m)	35.4	2.31 (2H, d, J = 9.0 Hz)	35.7	2.42 (1H, m) 2.09 (1H, m)
15	42.9	–	42.8	–	42.2	–	42.3	–	43.3	–	42.9	–
16	26.0	1.25 (3H, s)	26.2	1.26 (3H, s)	26.7	1.13(3H, s)	26.6	1.11 (3H, s)	26.4	1.23 (3H, s)	25.8	1.25 (3H, s)
17	21.5	1.29 (3H, s)	20.9	1.20 (3H, s)	19.8	1.17(3H, s)	19.9	1.15 (3H, s)	20.9	1.18 (3H, s)	21.6	1.27 (3H, s)
18	14.6	1.84 (3H, s)	14.4	1.86 (3H, s)	15.2	2.05 (3H, s)	15.1	2.03 (3H, s)	14.6	1.89 (3H, s)	14.6	1.96 (3H, s)
19	15.6	2.25 (1H, m) 1.66 (1H, t, J = 5.8 Hz)	16.3	2.23 (1H, m) 1.66(1H, t, J = 5.8 Hz)	16.2	2.34 (1H, m) 1.74 (1H, t, J = 6.2 Hz)	16.1	2.33 (1H, m) 1.74 (1H, t, J = 6.2 Hz)	10.8	1.81 (3H, s)	15.7	2.23 (1H, m) 1.66 (1H, m)
20	75.4	4.31(1H, d, J = 8.8 Hz) 4.03(1H, d, J = 8.8 Hz)	75.4	4.32(1H,d, J = 8.8 Hz) 4.05(1H,d, J = 8.8 Hz)	75.6	4.33 (1H, d, J = 8.4 Hz) 4.05 (1H, d, J = 8.4 Hz)	75.5	4.32 (1H, d, J = 8.4 Hz) 4.06 (1H, d, J = 8.4 Hz)	76.4	4.31 (1H, d, J = 8.5 Hz) 4.17 (1H, d, J = 8.5 Hz)	75.4	4.31 (1H, m) 4.04 (1H, d, J = 8.8 Hz)
1′	172.8	–	172.7	–	––	–	–	–	172.7	–	171.6	–
2′	73.7	4.61 (1H, br.s)	73.7	4.61 (1H, br.s)	–	–	–	–	73.6	4.64 (1H, br.s)	76.6	5.28 (1H, br.s)
3′	56.0	5.28 (1H, d, J = 8.8 Hz)	56.0	5.28(1H,br.d, J = 8.4 Hz)	–	–	–	–	56.1	5.26 (1H, d, J = 8.5 Hz)	54.1	5.61 (1H, br.d, J = 9.5 Hz)
4′	138.5	–	138.5	–	–	–	–	–	138.4	–	138.8	–
5′,9′	126.6	7.38 (2H, d, J = 7.0 Hz)	126.6	7.37 (2H, d, J = 7.7 Hz)	–	–	–	–	126.8	7.38 (2H, d, J = 7.2 Hz)	126.3	7.37 (2H, d, J = 7.3 Hz)
6′,8′	128.9	7.35(2H, t, J = 7.0 Hz)	128.8	7.34(2H, t, J = 7.7 Hz)	–	–	–	–	128.8	7.35 (2H, t, J = 7.2 Hz)	128.6	7.38 (2H, t, J = 7.3 Hz)
7′	128.0	7.31(1H, t, J = 7.0 Hz)	128.0	7.31(1H, t, J = 7.7 Hz)	–	–	–	–	128.0	7.32 (1H, t, J = 7.2 Hz)	127.8	7.32 (1H, t, J = 7.3 Hz)
10′	155.2	–	155.2	–	–	–	–	–	155.3	–	155.7	–
11′	80.1	–	80.1	–	–	–	–	–	80.2	–	80.3	–
11′-$(CH_3)_3$	28.1	1.28 (9H, s)	28.1	1.29 (9H, s)	–	–	–	–	28.2	1.36 (9H, s)	28.4	1.45 (9H, s)
1′	167.4	–	167.4	–	167.4	–	167.3	–	166.9	–	167.6	–
2′	129.2	–	129.3	–	129.4	–	129.5	–	129.1	–	129.1	–
3′,7′	130.3	8.15 (2H, d, J = 7.7 Hz)	130.3	8.15(2H, d, J = 7.7 Hz)	130.2	8.15 (2H, d, J = 7.7 Hz)	130.1	8.14 (2H, d, J = 7.7 Hz)	130.2	8.10 (2H, d, J = 7.7 Hz)	130.3	8.16 (2H, d, J = 7.7 Hz)
4′,6′	128.7	7.50 (2H, t, J = 7.7 Hz)	128.7	7.51 (2H, t, J = 7.7 Hz)	128.7	7.48 (2H, t, J = 7.7 Hz)	128.6	7.49 (2H, t, J = 7.7 Hz)	128.7	7.49 (1H, t, J = 7.7 Hz)	128.8	7.51 (1H, t, J = 7.7 Hz)
5′	133.6	7.60 (1H, t, J = 7.7 Hz)	133.6	7.61 (1H, t, J = 7.7 Hz)	133.7	7.60(1H, t, J = 7.7 Hz)	133.6	7.62 (1H, t, J = 7.7 Hz)	133.7	7.62 (1H, t, J = 7.7 Hz)	133.6	7.58 (1H, t, J = 7.7 Hz)

Table 2 (*Continued*)

Position	Larotaxel		Impurity II		Impurity I		Impurity III		Imputity IV		Imputity V	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
4-OAc	22.2, 169.7	2.37 (3H, s)	22.2, 169.8	2.38 (3H, s)	22.2, 170.1	2.28 (3H, s)	22.1, 170.2	2.27 (3H, s)	22.5, 170.1	2.37 (3H, s)	22.4, 169.5	2.57 (3H, s)
10-OAc	20.8, 169.6	2.21 (3H, s)	–	–	20.7, 169.5	2.21 (3H, s)	–	–	20.7 168.9	2.18 (3H, s)	20.8, 169.6	2.21 (3H, s)
7-OAc	–	–	–	–	–	–	–	–	21.1, 170.4	2.04 (3H, s)	–	–
1-OH	–	1.91 (1H, s)	–	–	–	–	–	–	–	1.74 (1H, s)	–	1.83 (1H, s)
2′-OH	–	3.33 (1H, br.s)	–	–	–	–	–	–	–	3.41 (1H, d, J = 3.8 Hz)	–	–
3′-NH	–	5.39(1H,br d, J = 9.5 Hz)	–	5.40(1H,br d, J = 9.1 Hz)	–	–	–	–	–	5.43 (1H, br.d, J = 9.0 Hz)	–	6.75 (1H, d, J = 8.4 Hz)
2‴-OH	–	–	–	–	–	–	–	–	–	–	–	3.00 (1H, br.s)
3‴-NH	–	–	–	–	–	–	–	–	–	–	–	5.56 (1H, br.d, J = 10.2 Hz)
1‴	–	–	–	–	–	–	–	–	–	–	165.8	–
2‴	–	–	–	–	–	–	–	–	–	–	73.3	4.31 (1H, m)
3‴	–	–	–	–	–	–	–	–	–	–	54.6	5.51 (1H, m)
4‴	–	–	–	–	–	–	–	–	–	–	137.7	
5‴,9‴	–	–	–	–	–	–	–	–	–	–	126.8	7.31 (2H, m)
6‴,8‴	–	–	–	–	–	–	–	–	–	–	128.8	7.42 (2H, m)
7‴	–	–	–	–	–	–	–	–	–	–	128.1	7.36–7.28 (1H, m)
10‴	–	–	–	–	–	–	–	–	–	–	155.3	–
11‴	–	–	–	–	–	–	–	–	–	–	79.9	–
11‴-$(CH_3)_3$	–	–	–	–	–	–	–	–	–	–	28.0	1.19 (9H, s)

s, singlet; d, doublet; t, triplet; m, multiplet; dd, double doublet; dt, double triplet; br.s, broad singlet; br.d, broad doublet.

that of Impurity III by 42 Da. In ^{1}H and ^{13}C NMR spectrum, the C10 acetyl proton signal and carbonyl signal were absent, which was present in larotaxel at 2.21 ppm and 169.5 ppm, respectively. The significant change was found in the ^{1}H chemical shift value of the C10 proton. Therefore, Impurity III was confirmed as 10-deacetyl-7,8-cyclopropyl baccatin III.

3.2.5. Impurity IV

The ESI mass spectrum of Impurity IV gave a sodium adduct $[M+Na]^+$ at m/z 914 and a protonated molecular ion $[M+H]^+$ at m/z 892, indicating that Impurity IV had a molecular mass more than that of larotaxel by 60 Da. In ^{1}H NMR spectrum, two new methyl proton signals were observed at 1.81 ppm and 2.04 ppm, and the C19 methylene proton signal of larotaxel was absent. Simultaneously, the chemical shift value of C7 proton had also changed significantly. In ^{13}C NMR spectrum, a new carbonyl signal was observed at 170.4 ppm. The results of DEPT spectrum also confirmed the absence of C19 methylene proton signal and the appearance of C7 methyl proton and C8 methyl proton signals. In HMBC spectrum, the C9 carbon signal was found to be correlated with the C8 methyl proton signal (Table 1). All of these spectral data confirmed that Impurity IV was 7-acetyl-8-methyl larotaxel.

3.2.6. Impurity V

The ESI mass spectrum of Impurity V gave a sodium adduct $[M+Na]^+$ at m/z 1117 and a protonated molecular ion $[M+H]^+$ at m/z 1095, indicating that Impurity V had a molecular mass more than that of larotaxel by 263 Da. The fragmentation pathway of the protonated molecular ion at m/z 1095 was obtained: m/z at 552, 544, 488, 344, 221 and 181 (Fig. 3). These fragment ions were same as the fragmentation pattern of the proposed structure given in Table 1. In ^{1}H NMR spectrum, the proton signals of one monosubstituted Benzene were observed at 7.31–7.42 ppm and one trimethyl proton signal was visible at 1.19 ppm. In ^{13}C NMR spectrum, two carbonyl signals were observed at 165.8 ppm and 155.3 ppm, and one methyl and two methine proton signals were found in high field, while these signals were invisible in larotaxel. So it was supposed that Impurity V was bissidechain larotaxel. To identify the position of the other side chain, ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H–^{1}H COSY, HSQC, and HMBC spectrum were used. Compared with larotaxel, no change was found in the ^{1}H and ^{13}C NMR signals of C1–C20. Thus the other side chain was not connected on the parent, instead, on the side chain of larotaxel. The ^{1}H NMR spectrum of Impurity V also showed the significant change in the ^{1}H chemical shift values of the C2′ proton and C3′ proton, which indicated the connection of the other side chain at 2′-OH position. The ^{1}H chemical shift values of the C2′ proton and C3′ proton of Impurity I were visible at 5.28 and 5.61 ppm, while in the ^{1}H NMR spectrum of larotaxel these were observed at 4.61 and 5.28 ppm. Moreover, the important correlation of the C1‴ signal and the C2′ proton signal was found in HMBC spectrum of Impurity V (Table 1). All of these spectral data confirmed that Impurity V was 2′,13-bissidechain larotaxel. It is a new compound which was unreported.

3.3. Formation of impurities

According to the structure of impurities and the synthetic process of larotaxel, the possible formation of impurities was proposed. Impurities I and II may be produced by the ester bond breaking of larotaxel, and Impurity III was caused by the ester bond breaking of Impurity I under acidic or basic conditions. It was proposed that the formation of Impurity IV was related to ethylacetate and acetic acid remained in larotaxel. Impurity V may be produced by the esterfication of position 2′ active hydroxy group.

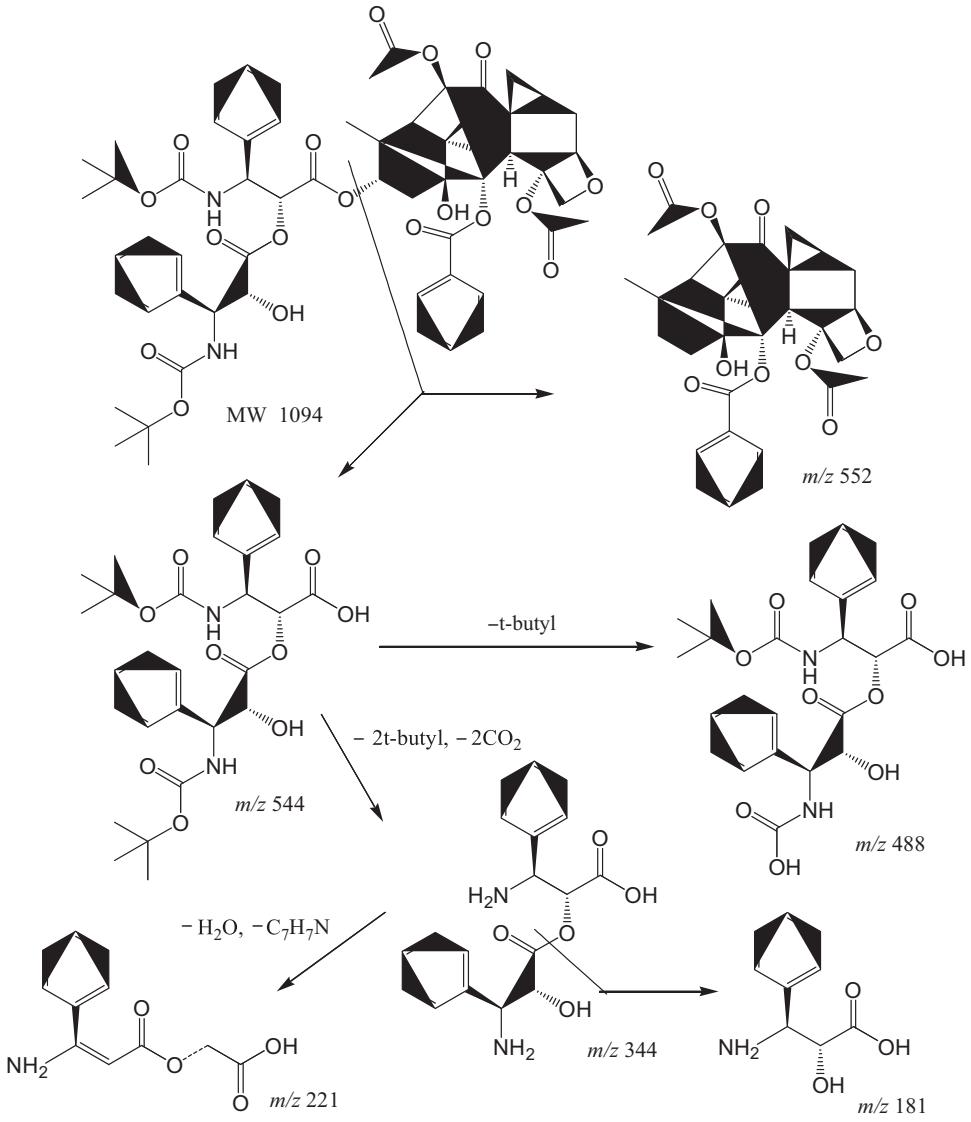


Fig. 3. Fragmentation pathway of Impurity V.

4. Conclusions

The process related impurities and major degradation of larotaxel were isolated from base degraded and stability samples of larotaxel using semi-preparative LC and characterized by NMR and MS. Impurities I, II and III were acid and base degradation products of larotaxel. Impurity IV and V were major impurities of 12-month stability samples. Moreover, Impurities I, II, IV and V were also process related impurities which were found in crude larotaxel. Impurities I and V are new compounds named as 7,8-cyclopropyl baccatin III and 2′,13-bissidechain larotaxel, respectively. The other three impurities were confirmed as10-deacetyl larotaxel (Impurity II), 10-deacetyl-7, 8-cyclopropyl baccatin III (Impurity III), 7-acetyl-8-methyl larotaxel (Impurity IV), respectively.

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