TWO NEW QUINAZOLINE-QUINOLINE ALKALOIDS FROM *PEGANUM NIGELLASTRUM*

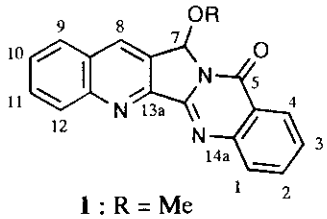
Zhong-Ze Ma,[a] Yoshio Hano,[a] Taro Nomura,*[a] and Ying-Jie Chen[b]
[a]Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan
[b]Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110015, P. R. China

Abstract - Two new quinazoline-quinoline alkaloids, luotonins E (**1**) and F (**2**), were isolated from the aerial parts of *Peganum nigellastrum* along with two known alkaloids 3-quinolinecarboxamide (**3**) and pegamine (**4**). The structures of luotonins E and F were determined to be the formulae (**1**) and (**2**), respectively, by spectroscopic and synthetic methods.

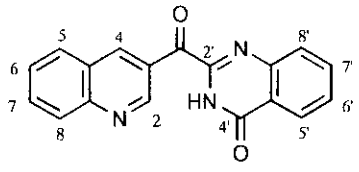
In our survey on the components of *Peganum nigellastrum* Bunge (Zygophyllaceae), Chinese medicinal plant "Luo-Tuo-Hao",[1] we have reported the structures of new alkaloid components luotonins A,[2] B,[2] C,[3] and D[3] isolated from the aerial parts of the plant. Furthermore, total synthesis of luotonin A, a cytotoxic pyrroloquinazolinoquinoline alkaloid against mouse leukemia P-388 cells, was achieved by three research groups, Ganesan's,[4] Kelley's,[5] and our groups.[6] *P. nigellastrum* is a rich source of alkaloid compounds, and it has been reported that the basic fraction exhibited anti-tumor activity.[7] Further extensive study on the alkaloid components of the plant has led to the isolation of two new alkaloids luotonins E (**1**) and F (**2**) along with two known alkaloids 3-quinolinecarboxamide (**3**)[8] and pegamine (**4**).[9] This paper deals with the isolation and the structure determination of the new alkaloids (**1**) and (**2**) by spectroscopic and synthetic methods.

P. nigellastrum was collected in Benshan area, the suburb of Wuhai city, Inner Mongolia, China. The dried aerial parts of the plant were extracted with ethanol and then the ethanol extract was divided into *n*-hexane, benzene, chloroform, acetone, and methanol soluble portions over Amberlite XAD-2. The *n*-hexane soluble portion was purified by silica gel column chromatography and preparative TLC to give luotonin F (**2**). Analogous separation procedures of the benzene and chloroform soluble portions gave 3-quinolinecarboxamide (**3**),[8] pegamine (**4**),[9] and luotonin E (**1**). The known alkaloids (**3**) and (**4**) were identified by comparing the physical data with the published data as well as by the NMR spectroscopic evidence. The compound (**3**) was the first isolation from natural resources.

Luotonin E (**1**) was obtained as pale yellow powder, mp 222 - 225 °C and gave a orange color with a Dragendorff reagent. The molecular formula, $C_{19}H_{13}N_3O_2$, was determined by high-resolution EIMS measurement. The IR spectrum of **1** showed absorption bands due to conjugated carbonyl group and aromatic ring. The UV spectrum exhibited absorption maxima at 213, 250, 301, 323sh, 337, and 352 nm and was closely similar to those of luotonins A[2] and B (**5**),[2] suggesting **1** to be a congener of



1 : R = Me
5 : R = H



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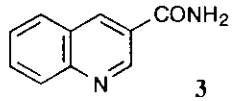

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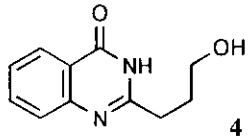

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Figure 1 Alkaloids from the aerial parts of *P. nigellastrum*

Table 1 ^{1}H and ^{13}C NMR data of **1, 2** and **5**

	1		**5**		**2**	
	^{13}C	^{1}H	^{13}C		^{13}C	^{1}H
C-1	128.9	8.09dd (1.5, 8.0)	129.2	C-2	150.5	9.92d (2.1)
C-2	134.9	7.85dt (1.5, 8.0)	135.1	C-3	126.6	
C-3	127.9	7.59dt (1.5, 8.0)	127.9	C-4	141.8	9.52d (2.1)
C-4	126.9	8.43dd (1.5, 8.0)	126.5	C-4a	126.4	
C-4a	122.3		121.9	C-5	129.6	8.05br d (8.0)
C-5	160.9		161.6	C-6	127.5	7.72t (8.0)
C-7	87.0	6.95s	80.9	C-7	132.8	7.93t (8.0)
C-7a	130.1		131.3	C-8	129.0	8.23br d (8.0)
C-8	133.2	8.52s	133.7	C-8a	149.3	
C-8a	129.0		128.9	C-2'	145.4	
C-9	128.6	8.00dd (1.5, 8.0)	128.6	C-4'	160.3	
C-10	128.8	7.72dt (1.5, 8.0)	128.8	C-4'a	123.1	
C-11	131.4	7.88dt (1.5, 8.0)	131.4	C-5'	126.7	8.43dd (1.5, 8.0)
C-12	130.9	8.48dd (1.5, 8.0)	130.9	C-6'	129.6	7.70dt (1.5, 8.0)
C-12a	150.4		150.3	C-7'	134.8	7.90ddd (1.5, 8.0, 8.6)
C-13a	150.5		150.4	C-8'	129.2	7.98dd (1.5, 8.6)
C-13b	151.5		150.9	C-8'a	147.1	
C-14a	149.0		149.5	C=O	183.8	
OMe	56.3	3.60s		3-NH		10.21br s

Measured in $CDCl_3$. Values in parentheses are coupling constants (*J* in Hz).

pyrroloquinazolinoquinoline alkaloids. The ^{1}H NMR spectrum of **1** was also similar to that of luotonin B (**5**) but was distinctive in that **1** showed the signal of a methoxyl group at δ 3.60 (3H, s). By comparision of the ^{1}H and ^{13}C NMR spectra of **1** with those of luotonin B (**5**), **1** was clear to be 7-*O*-monomethyl ether of **5** (7-methoxyluotonin A) (Table 1). Furthermore, the conversion of luotonin B (**5**) to **1** by a treatment with BF_3 etherate in a methanol solution[10] confirmed the structure of luotonin E.[11]

Luotonin F (**2**) was obtained as pale yellow needles, mp 238 - 240 °C and showed positive to the Dragendorff test. The molecular formula, $C_{18}H_{11}N_3O_2$, was determined by high-resolution EIMS measurement. The UV spectrum of **2** exhibited maxima at 212, 255, 303, 316sh, and 328sh nm. The IR spectrum of **2** disclosed the absorption bands due to aromatic and conjugated carbonyl groups. The ^{1}H

NMR spectrum of **2** displayed two sets of aromatic A_2B_2 type signals at δ 7.72, 7.93 (each 1H, t, J = 8.0 Hz), 8.05, 8.23 (each 1H, br d, J = 8.0 Hz); δ 7.70, 7.90 (each 1H, dt, J = 1.5 and 8.0 Hz), 7.98, 8.43 (each 1H, dd, J = 1.5 and 8.0 Hz), *meta*-coupled proton signals at δ 9.52 (d, J = 2.1 Hz), 9.92 (d, J = 2.1 Hz), and a broad signal at δ 10.21 (1H, NH). These proton signals suggested that compound (**2**) is consist of two alkaloid parts, a 3-substituted quinoline and a 2-substituted quinazolinone, such as **3** and **4**, respectively. Furthermore, the ^{13}C NMR spectrum of **2** indicated the presence of a carbonyl group (δ 183.9) in addition to the quinoline and quinazolinone moieties (Table 1). The C-H connectivities in the molecule were performed with the aid of the HMQC and HMBC spectra. The HMBC spectrum revealed a structure, in which the C-3 of the quinoline moiety is linked to the C-2 of quinazolinone moiety through a junction of the carbonyl group, that is 3-[(3*H*)-quinazolinone]carbonylquinoline (Figure 2). The structure of luotonin F was thus represented by the formula (**2**).

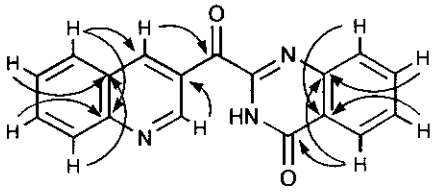
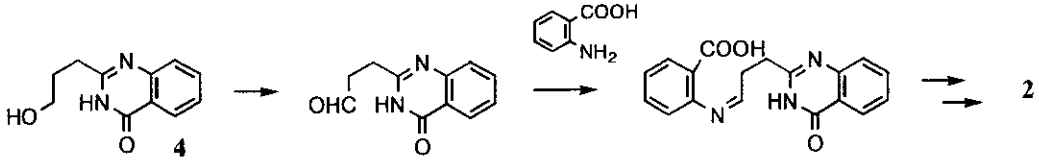


Figure 2 Significant HMBC correlations (J_{CCH} = 8 Hz) of **2**

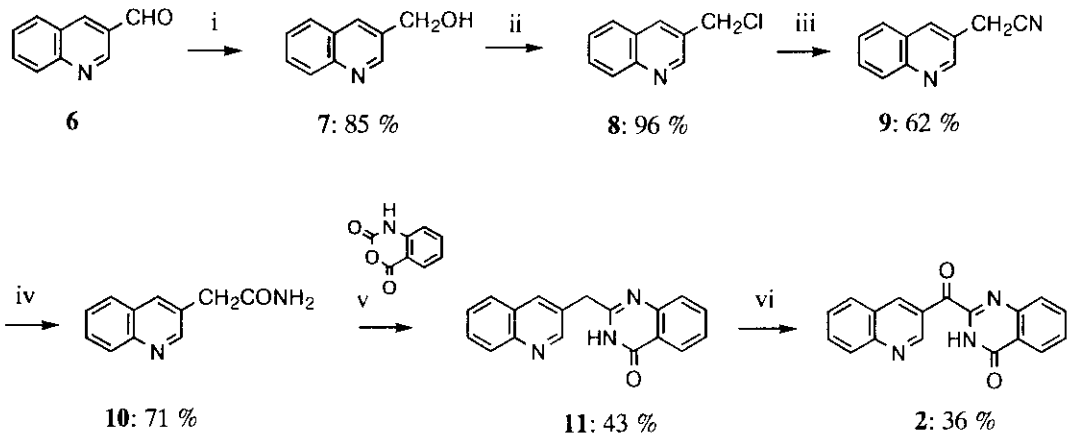


Scheme 1 A plausible biosynthetic route to luotonin F (**2**) from pegamine (**4**)

On the other hand, coexist of luotonin F (**2**) and pegamine (**4**) in the same source allowed to deduce a hypothetical biosynthetic route to **2** from **4** as shown in Scheme 1.

Furthermore, synthetic study of **2** aiming not only at its structural confirmation but also at securing quantity enough to evaluate its biological activities was examined as shown in Scheme 2.

3-Hydroxymethylquinoline (**7**) prepared by a reduction of 3-formylquinoline (**6**) with $NaBH_4$ was converted to a chloride (**8**, 96 % yield) which, in turn, was treated with KCN to provide a nitrile (**9**) in 62 % yield. Hydrolysis of **9** with sulfuric acid yielded an amide (**10**) in 71 % yield and then reaction of the amide (**10**) with isatoic anhydride[12] under heating at 200 - 210 °C for 2 h gave deoxoluotonin F (**11**) in 43 % yield. Finally, treatment of **11** with activated manganase dioxide under exposure to sunlight for 6 h afforded a ketone in 36 % yield. The physical and spectroscopic data of the product were superimposable to those of luotonin F. Thus, the structure of **2** was unambiguously established.



Scheme 2 Total synthesis of **2**: i, $NaBH_4$, MeOH; ii, $SOCl_2$, benzene; iii, KCN, KI, 80 % EtOH; iv, conc. H_2SO_4; v, isatoic anhydride, 200 - 210 °C; vi, MnO_2, $CHCl_3$, sunlight

EXPERIMENTAL

Melting points were determined by Yanaco micro-melting point apparatus MP-500V and are uncorrected. Optical rotation was recorded on a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a JASCO FTIR 300 spectrophotometer and UV spectra were recorded on a Shimadzu 265 UV spectrophotometer. NMR spectra were recorded on a JEOL JNM EX-400 FTNMR spectrometer. MS spectra were recorded on a JEOL JMS DX-303 spectrometer and electro-spray ionization (ESI) MS spectra were recorded on a LCQ mass spectrometer (Finigan MAT). Wakogel C-200 and B-5 FM (Silica gel, Wako Pure Chemical Co., Ltd, Osaka, Japan) were used for column chromatography and TLC, respectively. HPLC was carried out on a SSC Flow System E-3100 (Senshu Scientific Co., Ltd., Tokyo, Japan) equiped with a SSC-3000B UV monitor. SSC Senshu Pack Silica 4251-N (10ϕ x 250 mm) and Aquasil SS-762N (20ϕ x 250 mm) were used for HPLC at a flow rate of 2 mL/min.

Plant material

The aerial parts of *P. nigellastrum* Bunge was collected in Beishan area, the suburbs of Wuhai city, Inner Mongolia, China, in August 1994, and identified by Prof. Shi-Rui Xing, Ningxia Institute for Drug Control. The specimen was deposited in Shenyang Pharmaceutical University.

Extraction and isolation

The dried aerial parts of *P. nigellastrum* (5 kg) was repeatedly extracted three times with 95% EtOH (8 L) under reflux for 2 h. The EtOH solution was evaporated *in vacuo* to give a residue (700 g). The EtOH extract (350 g) was fractionated over Amberlite XAD-2 by successive elutions with *n*-hexane, C_6H_6,

$CHCl_3$, Me_2CO, and MeOH to give each soluble parts. The *n*-hexane part (17 g) was chromatographed over silica gel (250 g) using *n*-hexane increasing amount of EtOAc to prepare frs. 1-72 (200 mL/fr.). The combined frs. 47 - 51 eluted with *n*-hexane - EtOAc (2 : 1) was purified by preparative TLC [$CHCl_3$ - Me_2CO (5 : 1)] and HPLC [$CHCl_3$ - EtOAc (3 : 1)] to give luotonin F (**2**, 1.2 mg). The C_6H_6 part (15 g) was subjected to silica gel (250 g) column chromatography using *n*-hexane increasing amount of EtOAc as an eluent to prepare frs. 1 - 300 (300 mL/fr.). The combined frs. 251 - 310 (5.37 g) eluted with *n*-hexane - EtOAc (1 : 1 - 1 : 2) was purified by preparative TLC [$CHCl_3$ - MeOH (5 : 1)] and HPLC [$CHCl_3$ - MeOH (8 : 1)] to give 3-quinolinecarboxamide (**3**, 8 mg)[8] and pegamine (**4**, 15 mg).[9] The $CHCl_3$ part (30 g) was chromatographed over silica gel (300 g) using C_6H_6 increasing amount of Me_2CO as an eluent to prepare frs. 1 - 315 (250 mL/fr.). The combined frs. 9-13 eluted with C_6H_6-Me_2CO (20 : 1) was purified by preparative TLC [$CHCl_3$ - Me_2CO (5:1)] to give luotonin E (**1**, 0.8 mg).

Luotonin E (1)

Compound (**1**) was obtained as a pale yellow crystalline powder, mp 222 - 225 °C, and positive to Dragendorff test on a TLC plate. $[\alpha]_D$ 0° (*c* = 0.016, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 213 (4.67), 250 (4.66), 301 (4.13), 323 (sh, 4.21), 337 (4.28), 352 (4.18). IR ν_{max} (KBr) cm^{-1}: 1682, 1634, 1604, 1465, 1350, 1323, 1080, 769, 691. ESIMS: *m/z* 316 $(M+H)^+$. HR- EIMS: *m/z* 315.1000 (M^+, $C_{19}H_{13}N_3O_2$, requires 315.1008).

Luotonin F (2)

Compound (**2**) was obtained as pale yellow needles, mp 238 - 240 °C, and positive to Dragendorff test on a TLC plate. UV λ_{max} (MeOH) nm (log ε): 212 (4.40), 255 (3.98), 303 (3.83), 316 (sh, 3.79), 328 (sh, 3.70). IR ν_{max} (KBr) cm^{-1}: 3434, 1661, 1615, 1599, 1467, 1444, 1335, 1298, 1239, 1157, 1131, 975, 899, 876, 827, 781, 752. ESIMS: *m/z* 302 $(M+H)^+$. HR-EIMS: *m/z* 301.0839 (M^+, $C_{18}H_{11}N_3O_2$, requires 301.0852).

Synthesis of Luotonin E (1)

A solution of luotonin B (**5**, 5 mg) and BF_3 etherate (0.5 mL) in methanol (10 mL) was refluxed for 2 h. After the solvent was removed *in vacuo*, the residue was purified by preparative TLC [solvent system, $CHCl_3$ - Me_2CO (10 : 1)] to give **1** (3.6 mg, 70 % yield). The physical, NMR, and MS data were identical with those of natural **1**.

3-Hydroxymethylquinoline (7)

A mixture of 3-formylquinoline (**6**, 256mg, 1.63 mmol) and $NaBH_4$ (150 mg, 3.95 mmol) in methanol (15 mL) was stirred at rt for 2 h. After usual work-up, the reaction product was purified by preparative TLC [$CHCl_3$ - Me_2CO (2 : 1)] to afford **7** (233 mg, 85 %), colorless crystals ($CHCl_3$ - Me_2CO), mp 83 - 85 °C (lit.,[13] 83.5 - 84 °C). ^1H NMR (400 MHz, $CDCl_3$): δ 4.73 (2H, s), 7.34 (1H, dt, *J* = 1.2 and 8.4 Hz), 7.48 (1H, dt, *J* = 1.2 and 8.4 Hz), 7.53 (1H, d, *J* = 8.4 Hz), 7.89 (1H, d, *J* = 8.4 Hz), 7.93 (1H, d, *J* = 2.1 Hz), 8.64 (1H, d, *J* = 2.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 61.0, 125.9, 126.8, 126.9, 127.3, 128.4, 133.0, 133.4, 145.8, 148.9. ESIMS: *m/z* 160 $(M+H)^+$.

3-Chloromethylquinoline (8)

A solution of **7** (122 mg, 0.77 mmol) and thionyl chloride (0.5 mL, 6.95 mmol) in dry benzene (20 mL) was refluxed for 2 h. After benzene and an excess thionyl chloride were removed *in vacuo*, a 10 mL of water was added to the residue and further basified with 10% NaOH aqueous solution. Extraction of the solution with chloroform followed by evaporation of the chloroform afforded a pale yellow powder which was purified with preparative TLC [$CHCl_3$ - Me_2CO (10 : 1)] to give **8** (136 mg, 96 %), colorless oil (lit.,[14] mp 33 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.78 (2H, s), 7.58 (1H, dt, *J* = 1.2 and 8.1 Hz), 7.74 (1H, dt, *J* = 1.2 and 8.4 Hz), 7.83 (1H, d, *J* = 8.1 Hz), 8.13 (1H, d, *J* = 8.4 Hz), 8.17 (1H, d, *J* = 2.1 Hz), 8.94 (1H, d, *J* = 2.1 Hz). EIMS: *m/z* (rel. int.) 179, 177 (M^+, 11 and 35, respectively), 142 (M^+-Cl, 100).

3-Cyanomethylquinoline (9)

A mixture of **8** (225 mg, 1.27 mmol), potassium cyanide (200 mg, 3.08 mmol) and potassium iodide (200 mg, 1.20 mmol) in 80% ethanol (10 mL) was refluxed for 3 h. After usual work-up, the reaction product was purified by preparative TLC [$CHCl_3$ - Me_2CO (10 : 1)] to give **9** (134 mg, 62 %), colorless needles ($CHCl_3$ - Me_2CO), mp 82 - 84 °C (lit.,[15] 84 - 85 °C). IR ν_{max} (KBr) cm^{-1} : 2253. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.94 (2H, s), 7.58 (1H, dt, *J* = 1.4 and 8.2 Hz), 7.73 (1H, dt, *J* = 1.4 and 8.4 Hz), 7.81 (1H, d, *J* = 8.2 Hz), 8.10 (1H, d, *J* = 8.6 Hz), 8.16 (1H, d, *J* = 2.0 Hz), 8.80 (1H, d, *J* = 2.0 Hz). ESIMS: *m/z* 169 $(M+H)^+$.

3-Quinolineacetamide (10)

A mixture of **9** (50 mg, 0.30 mmol), conc. sulfuric acid (1 mL), and water (1 drops) was heated on a water bath for 30 min. The reaction mixture was poured into ice-water and then basified with 20% sodium hydroxide aqueous solution. After extraction with chloroform, the extract was purified by preparative TLC [$CHCl_3$ - MeOH (5 : 1)] to give **10** (39.6 mg, 71 %), colorless needles ($CHCl_3$ - Me_2CO), mp 198 - 200 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.78 (2H, s), 7.57 (1H, dt, *J* = 1.4 and 8.4 Hz), 7.72 (1H, dt, *J* = 1.4 and 8.4 Hz), 7.81 (1H, dd, *J* =1.4 and 8.4 Hz), 8.10 (1H, d, *J* = 8.6 Hz), 8.11 (1H, d, *J* = 2.0 Hz), 8.84 (1H, d, *J* = 2.0 Hz). ESIMS: *m/z* 187$(M+H)^+$. HR-EIMS: m/z 186.0787 (M^+, $C_{11}H_{10}N_2O$, requires 186.0793).

3-[(3*H*)-Quinazolinone]methylquinoline (**11**)

A mixture of **10** (20 mg, 0.11 mmol) and isatoic anhydride (100 mg, 0.61 mmol) was heated at 200 - 210 °C for 2 h. Afetr cooling to rt, the reaction mixture was purified by preparative TLC [$CHCl_3$ - Me_2CO (2 : 1)] to give **11** (13.4 mg, 43%), colorless needles ($CHCl_3$ - Me_2CO), mp 246 - 248 °C. IR ν_{max} (KBr) cm^{-1}: 3404, 1677, 1610, 1496, 1468, 1449, 1397, 1334, 1162, 1137, 770, 757. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.25 (2H, s), 7.50 (2H, dt, *J* = 1.4 and 8.4 Hz), 7.67 (1H, dt, *J* = 1.4 and 8.4 Hz), 7.73 (2H, d, *J* = 8.4 Hz), 7.79 (1H, dt, *J* = 1.4 and 8.4 Hz), 8.06 (1H, d, *J* = 8.6 Hz), 8.22 (1H, d, *J* = 2.1 Hz), 8.27 (1H, dd, *J* = 1.4 and 8.4 Hz), 9.01 (1H, d, *J* = 2.1 Hz), 11.65 (1H, br s, NH). ESIMS: *m/z* 288 $(M+H)^+$. HR-EIMS: 287.1051 (M^+, $C_{18}H_{13}N_3O$, requires 287.1059).

Synthesis of Luotonin F (2)

A mixture of **11** (10 mg, 0.035 mmol) and active manganese dioxide (52 mg, 0.60 mmol) in chloroform (10 mL) was stirred for 6 h under exposure to sunlight. After usual work-up, the reaction mixture was purified by preparative TLC [$CHCl_3$ - Me_2CO (2 : 1)] to give a product (3.8 mg, 36 %) as pale yellow needles. The mp, UV, IR, ^1H NMR and MS spectra data were completely agreement with those of natural specimen (**2**).

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11. In our previous paper,[2] we described a possibility that luotonin B (**5**) is an artifact from luotonin A, because of no optical activity. Luotonin E (**1**, 7-methoxyluotonin A) also showed no optical activity. However, the isolation of **1** was carried out under the condition that methanol has not been used at all as a solvent throughout the separation procedures (see EXPERIMENTAL). This fact indicated that both **1** and **5** might be genuine products in the title plant.
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