STRUCTURAL STUDIES ON NEW DEPSIPEPTIDE ANTIBIOTICS, VARIAPEPTIN AND CITROPEPTIN

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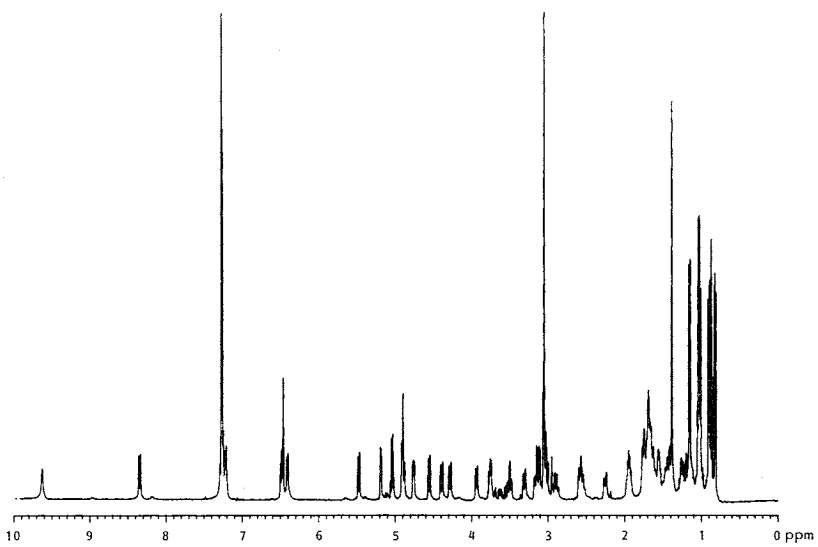
Variapeptin and citropeptin were found as novel hexadepsipeptide antibiotics produced by *Streptomyces variabilis* and *Streptomyces flavidovirens*, respectively. Their structures were elucidated by NMR spectral analysis including a variety of 2D techniques. Variapeptin and citropeptin are structurally related to azinothricin and A83586C, respectively.

In the course of our screening program for new antitumor antibiotics, variapeptin and citropeptin were found to be produced by *Streptomyces variabilis* and *Streptomyces flavidovirens*, respectively. The fermentation, isolation, characterization and biological properties of these antibiotics have been reported in the previous papers[1,2]. These antibiotics were elucidated by NMR techniques to be new cyclic hexadepsipeptides related to azinothricin[3] and A83586C[4]. We wish to report herein the structural elucidation of variapeptin and citropeptin.

Variapeptin

The ^{1}H and ^{13}C NMR spectra of variapeptin were analyzed using a variety of 2D NMR techniques such as COSY, NOESY and heteronuclear multiple-bond correlation (HMBC). The ^{1}H NMR spectrum of variapeptin is shown in Fig. 1.

Fig. 1. The ^{1}H NMR spectrum of variapeptin in $CDCl_3$ (500 MHz).



The ^{13}C and ^{1}H NMR spectra of variapeptin summarized in Table 1 showed the presence of seven amide or ester carbonyl groups (δ_C 177.0, 174.2, 173.6, 171.9, 170.5, 170.1 and 168.9) and six *N*-methine groups (δ_C 54.9, 52.5, 52.5, 52.0, 51.7 and 50.6) assignable to the α-methines of amino acid residues. These data suggest that variapeptin is a peptide antibiotic consisting of six amino acids and one carboxylic acid residue.

The partial structures (a to g) with the proton spin networks in variapeptin indicated by bold lines were elucidated by ^{1}H-^{1}H and ^{1}H-^{13}C COSY as shown in Fig. 2. The residual isolated proton and quaternary carbon signals were as follows; the above mentioned seven carbonyls, a ketal or hemiketal carbon (δ_C 99.1), a quaternary oxycarbon (δ_C 77.0), a methyl group (δ_C 19.9, δ_H 1.35), and two exchangeable protons (δ_H 9.48 and 4.28).

The connections of protonated carbons to quaternary carbons and/or hetero atoms were analyzed by NOESY and HMBC[5,6] spectroscopy, which detected C-H long range couplings separated by two or three bonds. The results summarized in Fig. 2 reveal the presence of each one molecule of *N*-methylphenylalanine (c), *N*-hydroxyalanine (d), 3-hydroxyleucine (f) and serine (a).

The remaining amino acid moieties were identified as two piperazic acid residues (b and e) by close spectral similarity to the same moieties in A83586C (see Table 1). The NOE observed between a methyl proton (21-H, δ 1.12, unit d) and an exchangeable proton (δ 9.48), which was coupled with no carbon in the HMBC spectrum, revealed the presence of an *N*-hydroxy group in the alanine moiety. The spectral data of this moiety (d) is similar to those of the corresponding moiety in azinothricin and A83586C.

Fig. 2. Partial structures of variapeptin.

The solid-line arrows indicate ^{1}H-^{13}C long range couplings detected by HMBC and dashed line arrows indicate NOE.

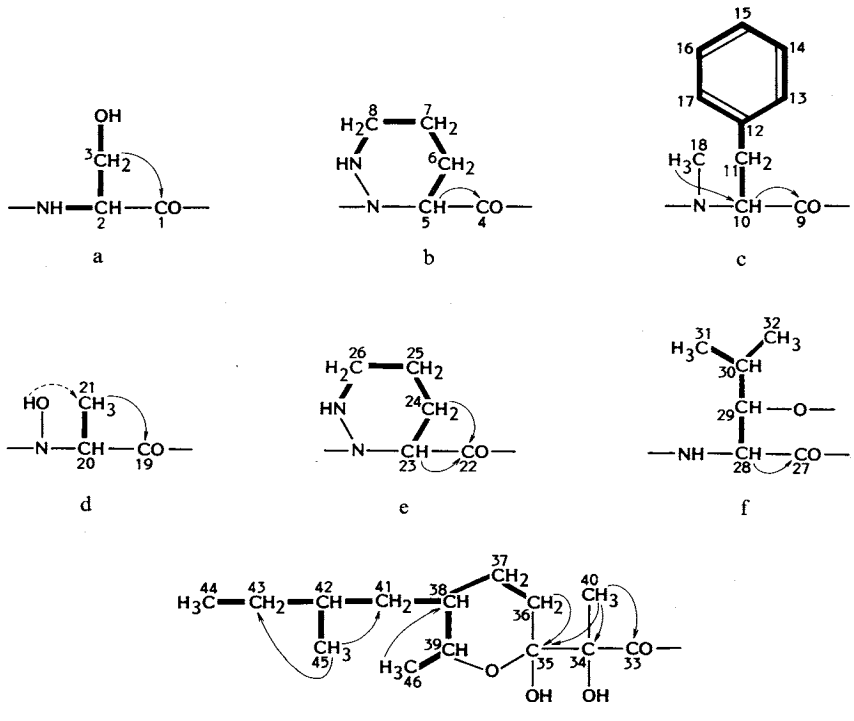


Table 1. ^{13}C and ^{1}H NMR assignments for variapeptin and A83586C in $CDCl_3$.

Position	Variapeptin		A83586C[a]		Position	Variapeptin		A83586C[a]	
	δ_C	δ_H	δ_C	δ_H		δ_C	δ_H	δ_C	δ_H
Ser					24	24.1	1.92, 2.23	24.22	1.88, 2.27
1	170.1				25	21.2	1.45, 1.61	21.36	1.46, 1.62
2	52.5	4.73			26	46.0	2.87, 3.13	45.79	2.98, 3.17
3	60.7	3.47, 4.52			26-NH		4.36		4.43
3-OH		4.28			3-OH-Leu				
2-NH		6.38			27	170.5		171.04	
Pip					28	54.9	4.86	54.82	4.92
4	168.9		169.63		29	78.9	5.45	78.41	5.45
5	52.5	5.16	52.43	5.20	30	29.4	1.92	29.38	1.73
6	24.4	1.67, 2.56	24.52	1.72, 2.56	31	19.9	0.87	19.50	0.72
7	21.4	1.53, 1.65	21.52	1.56	32	14.9	0.99	14.77	0.83
8	47.8	2.52, 3.28	47.94	2.62, 3.31	28-NH		8.32		8.24
8-NH		3.93		3.90	Side chain				
N-Me-Phe					33	177.0			
9	171.9				34	77.0			
10	52.0	6.45			35	99.1			
11	33.8	3.00, 3.09			36	27.7	1.64, 1.73		
12	136.5				37	24.0	1.39, 1.73		
13, 17	129.3	7.23			38	39.5	1.08		
14, 16	128.2	7.23			39	71.6	3.75		
15	126.7	7.18			40	19.9	1.35		
18	29.3	3.02			41	38.5	1.01		
N-OH-Ala					42	31.1	1.39		
19	173.6				43	30.9	1.15, 1.24		
20	50.6	5.01			44	11.5	0.84		
21	12.9	1.12			45	18.6	0.79		
N-OH		9.48		9.82	46	19.2	1.00		
Pip									
22	174.2		173.55						
23	51.7	4.87	51.67	4.93					

[a] Data taken from ref 4.

With regard to the acyl side chain, the sequence from C-36 to C-44 in unit g was also elucidated as shown in Fig. 2 by COSY and HMBC. The linkages around the quaternary carbons C-33, C-34 and C-35 were established by analyzing the C-H long range couplings between methyl (C-40) and methylene protons (C-36) and these quaternary carbons. The ether bond between C-35 and C-39 was confirmed by their similar C-13 chemical shifts to those of the corresponding carbons in azinothricin. The *J* values between 37-H and 38-H were 9.1 Hz, indicating equatorial configuration of the C-6-alkyl unit on C-38.

The amide bonds at C-4, C-9, C-19 and C-22 and the ester bond at C-1 were assigned as shown in Fig. 3 by HMBC. It could not be verified by HMBC that the amide bond at C-27 was combined to piperazic acid; the NOE observed between 28-NH (δ 8.32) and 26-H (δ 3.13) revealed that the piperazic acid residue is connected to 3-hydroxyleucine. The NOE observed between an *N*-hydroxy proton (δ 9.48) and the α-methine proton (23-H, δ 4.87) of piperazic acid reveals that the C-22 carbonyl group binds to *N*-hydroxyalanine.

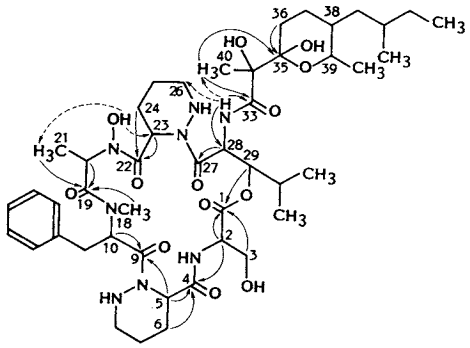
Thus it is concluded that the planar structure of variapeptin is as shown in Fig. 6.

Citropeptin

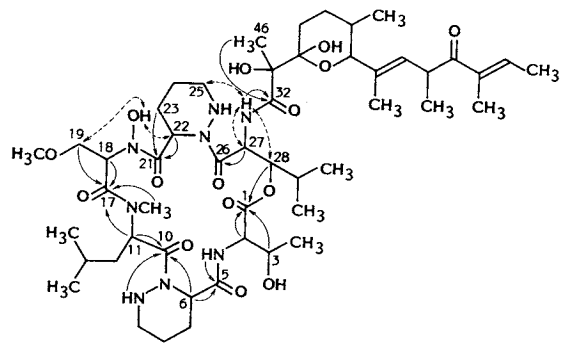
The structure of citropeptin was elucidated by the similar method as for applied to valiapeptin. The

Fig. 3. Amide and ester bond assignments in variapeptin and citropeptin.

The solid line and dashed line arrows are the same as those in Fig. 1.

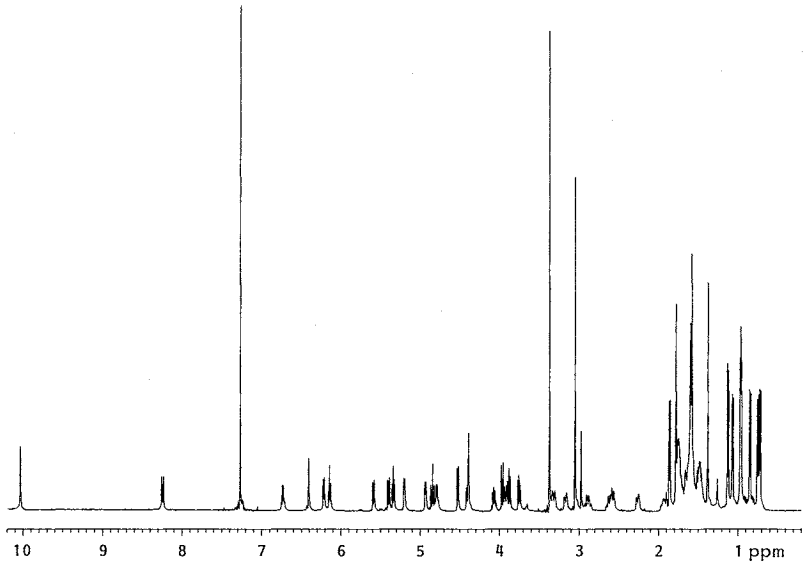


Variapeptin



Citropeptin

Fig. 4. The ^{1}H NMR spectrum of citropeptin in $CDCl_3$ (500 MHz).



^{1}H NMR spectrum of citropeptin is shown in Fig. 4. The ^{13}C and ^{1}H NMR spectral data were summarized in Table 2.

The structural fragments with isolated proton spin networks in citropeptin (a′ to g′ in Fig. 5) were elucidated by ^{1}H-^{1}H and ^{1}H-^{13}C COSY and homonuclear Hartmann-Hahn (HOHAHA)[7] spectroscopy which proved the arrangement of the methylene protons in piperazic acids, and the connectivities of the thus verified proton spin systems with quaternary carbons and hetero atoms were analyzed by NOESY and HMBC. The results summarized in Fig. 5 reveal the presence of threonine (a′), two piperazic acid residues (b′ and e′), *N*-methylleucine (c′), *O*-methylserine (d′) and 3-hydroxyleucine (f′).

The structure of the side chain containing six quaternary carbons was also elucidated as shown in Fig. 5 by NOESY and HMBC. The NOE observed between 38-H (δ 3.96) and 34-OH (δ 6.40) indicates that C-34 is connected to C-38 through an ether linkage. The geometric configurations at C-39 and C-43

Table 2. ^{13}C and ^{1}H NMR assignments for citropeptin in $CDCl_3$.

Position	δ_C	δ_H	Position	δ_C	δ_H
Thr			3-OH-Leu		
1	170.3		26	170.7	
2	56.0	4.52	27	54.5	4.84
3	64.4	4.79	28	78.2	5.39
4	18.6	1.06	29	29.3	1.73
2-NH		6.21	30	19.0	0.74
3-OH		4.39	31	14.6	0.84
Pip			27-NH		8.24
5	169.6		Side chain		
6	52.1	5.20	32	176.6	
7	24.1	2.57, 1.72	33	76.9	
8	21.1	1.62, 1.55	34	99.6	
9	47.6	3.31, 2.61	35	27.6	1.75, 1.53
9-NH		3.92	36	26.9	1.65, 1.62
N-Me-Leu			37	32.2	1.44
10	172.3		38	81.8	3.96
11	50.0	6.14	39	133.0	
12	36.2	1.75, 1.50	40	129.1	5.58
13	24.4	1.46	41	38.0	4.07
14	22.4	0.96	42	203.0	
15	22.4	0.95	43	136.7	
16	29.5	3.04	44	136.4	6.73
N-OH-*O*-Me-Ser			45	14.5	1.85
17	171.4		46	19.8	1.37
18	53.9	5.33	47	17.3	0.71
19	68.2	3.88, 3.75	48	11.8	1.57
20	58.8	3.37	49	18.8	1.12
18-NOH		10.03	50	11.1	1.77
Pip			33-OH		2.97
21	173.5		34-OH		6.40
22	51.1	4.93			
23	23.8	2.25, 1.94			
24	20.8	1.64, 1.50			
25	45.7	3.16, 2.88			
25-NH		4.40			

were determined to be 39*E* and 43*E* by the NOEs observed between 41-H (δ 4.07) and 48-H (δ 1.57) and between 45-H (δ 1.85) and 50-H (δ 1.77) in a rotaing-frame Overhauser enhancement spectroscopy (ROESY)[8] spectrum.

The amide bonds at C-5, C-10, C-17 and C-32 and the ester bond at C-1 were assigned as shown in Fig. 3 by HMBC. The NOE observed between the *N*-hydroxyl proton (δ 10.03) and the α-methine proton (22-H, δ 4.93) of the piperazic acid residue reveals that the C-21 carbonyl group binds to the *N*-hydroxy-*O*-methylserine residue with an amide linkage. Therefore, the remaining amide bond was elucidated to be formed between the C-27 carbonyl group in the 3-hydroxyleucine moiety and the piperazic acid residue. This linkage was corroborated by the NOE observed between 25-H (δ 2.88) and 27-NH (δ 8.24) as shown in Fig. 3.

Thus it is concluded that the structure of citropeptin is as shown in Fig. 6 except for the stereochemistry. Based on the similar specific rotation and ^{13}C chemical shift to those of A83586C ($[\alpha]_D^{25}$ +116.1°, *c* 0.2, $CHCl_3$), the stereochemistry including the absolute configuration of citropeptin is presumably identical with those of A83586C and azinothricin. Citropeptin differs from azinothricin in containing *N*-methylleucine

Fig. 5. Partial structures of citropeptin.

The solid line and dashed line arrows are the same as those in Fig. 1.

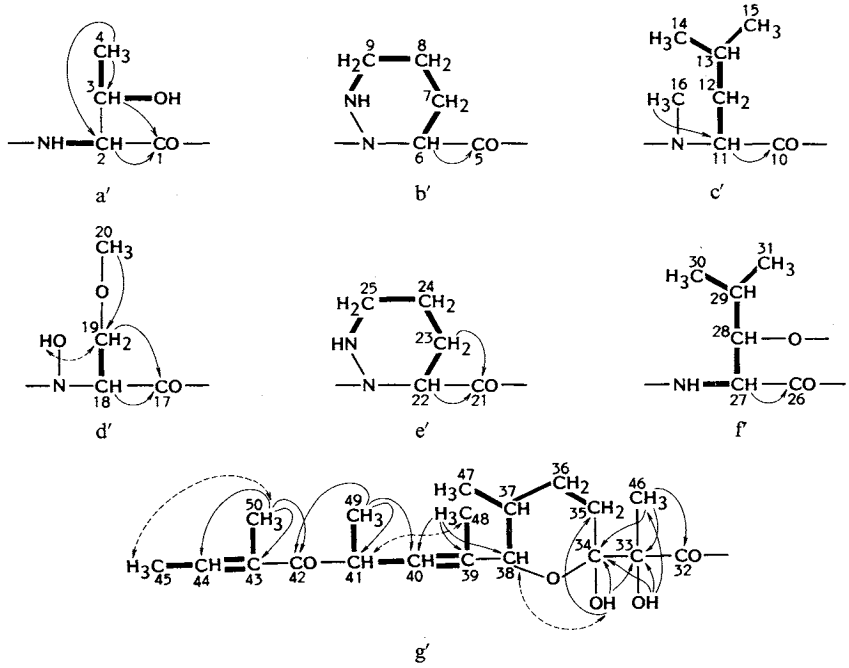
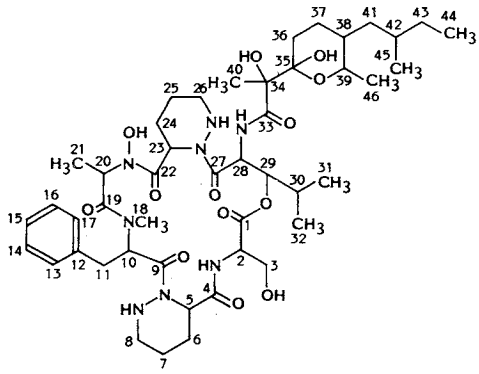
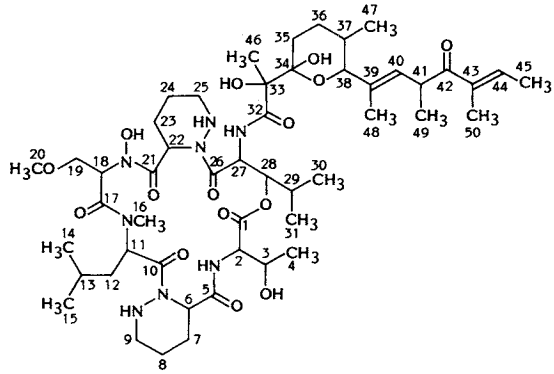


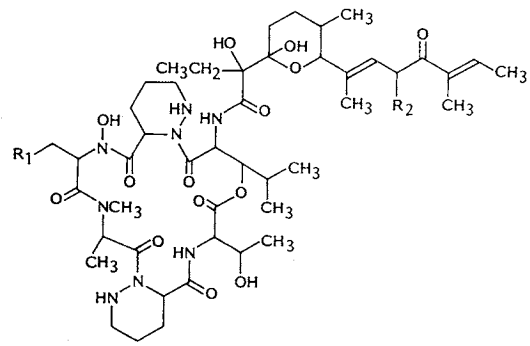
Fig. 6. Structures of variapeptin, citropeptin, azinothricin and A83586C.



Variapeptin



Citropeptin



Azinothricin $R_1=OCH_3$ $R_2=CH_2CH_3$

A83586C $R_1=H$ $R_2=CH_3$

and methyl groups (C-46, C-49) instead of *N*-methylalanine and ethyl groups, and from A83586C in containing *O*-methylserine, *N*-methylleucine and a methyl group (C-46) instead of *N*-hydroxyalanine, *N*-methylalanine and an ethyl group.

Experimental

NMR

The NMR spectra were obtained in $CDCl_3$ solution at 27°C on a Jeol GX-500 spectrometer. Chemical shifts were given in ppm using TMS as an internal standard.

Variapeptin

S. variabilis K2912 was cultured at 27°C for 4 days in a 50-liter jar fermenter containing 25 liters of a medium consisting of glucose 2.5%, soybean meal 1.5%, dry yeast extract 0.2% and $CaCO_3$ (pH 7.0).

The culture broth (50 liters) was centrifuged and the collected cake was extracted with acetone. The acetone extract was concentrated *in vacuo* to dryness, and then subjected to silica gel column chromatography (4 × 40 cm). The column was washed with $CHCl_3$ and the active substance was eluted with $CHCl_3$ - MeOH (30 : 1). Further purification was achieved by Sephadex LH-20 column chromatography (4 × 60 cm) with MeOH to give pure variapeptin (100 mg).

The physico-chemical properties of variapeptin were as follows: MP 158°C. *Anal* Calcd for $C_{46}H_{72}N_8O_{13}$: C 58.46, H 7.98, N 11.86, O 22.01. Found: C 58.01, H 7.82, N 12.07, O 22.10. $[\alpha]_D^{25}$ +128° (*c* 0.5 MeOH). UV λ_{max}^{MeOH} nm (ε) 222 (10,000). FD-MS *m/z* 945 (M+H). IR ($CHCl_3$) cm^{-1} 3400, 1730, 1660, 1640.

Citropeptin

S. flavidovirens K3619 was cultured at 27°C for 5 days in a 50-liter jar fermenter containing 25 liters of a medium consisting of glucose 2.5%, soybean meal 1.5%, dry yeast 0.2% and $CaCO_3$ (pH 7.0).

The whole broth (50 liters) was filtered through Celite and the mycelial cake was extracted with acetone. After being evaporated *in vacuo*, the extract was partitioned between EtOAc and water. The organic layer was concentrated to dryness, and then subjected to silica gel column chromatography. Development of the column (5 × 60 cm) with $CHCl_3$ - EtOH (50 : 1) gave an active fraction, which was evaporated to dryness and the residue was applied to the second silica gel column (4 × 50 cm). The crude active material eluted with $CHCl_3$ - EtOAc (2 : 1) was concentrated *in vacuo* and chromatographed on a Sephadex LH-20 column (2.5 × 50 cm) with MeOH. The active eluate was evaporated to dryness and crystallized from EtOH to yield 270 mg colorless needles of citropeptin: MP 109~111°C. *Anal* Calcd for $C_{50}H_{82}N_8O_{15}$: C 58.01, H 7.98, N 10.82, O 23.18. Found: C 58.11, H 7.88, N 10.77, O 23.00. $[\alpha]_D^{25}$ +113° (*c* 0.5, $CHCl_3$). UV λ_{max}^{MeOH} nm (ε) 230 (sh, 14,000). FD-MS *m/z* 1,035 (M+H), 1,057 (M+Na). IR ($CHCl_3$) cm^{-1} 3400, 2950, 1730, 1665, 1495, 1460, 1390, 1315, 1280, 1120, 1005.

Acknowledgments

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