Chiral Separation of Glycidyl Selenide and Glycidyl Sulfide Racemates on Cellulose-Based Chiral Stationary Phases

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Abstract

In recent years, a growing interest has been paid to glycidyl selenide and glycidyl sulfide racemic compounds for their importance in the life science field. In this study, cellulose-based chiral stationary phases are employed for the separation of glycerin selenium and glycerin sulfur racemates. Most analytes obtain satisfactory separation. In order to optimize the resolution of racemates, mixtures of *n*-hexane with different alcohols are used as mobile phases. The structural features of these racemic compounds affecting chiral discrimination are discussed in detail. The results in this study suggest that the chiral recognition mechanism for these racemic compounds involve two factors: (*a*) the substitution residue on a nonchiral atom can play a direct or indirect effect during chiral discrimination and (*b*) the competition between hydrogen-bonding and π–π interaction exists for compounds containing both the hydroxyl and aromatic group at the same time. The two interactions play an opposite role in the chiral discrimination process.

Introduction

Cellulose-based chiral stationary phases (CSPs) are the most widely used stationary phases for enantioseparations. In the past ten years, two research groups led by Okamoto and Ichida (1,2) initialized a series of investigations on cellulose ester and carbamate derivatives coated on silica gel. A wide range of polysaccharide-based CSPs for high-performance liquid chromatography (HPLC) is now commercially available for the separation of various enantiomers (3). Among them, cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) is one of the best derivatives having a high chiral recognition ability. In cellulose phenylcarbamates the polar carbamate group is the most important adsorption site that can interact with chiral compounds via hydrogen-bonding or dipole–dipole interaction. However, the π–π interaction between the aromatic groups of chiral compounds and the phenyl groups of the CSP may also play an important role. In order to reveal the mechanism of chiral recognition at a molecular level, more information is still needed. In this study, the optical resolution of 14 racemic glycerin selenium compounds and five racemic glycerin sulfur compounds having a similar structure was obtained on cellulose-based CSPs. The structural features of these racemic compounds affecting the chiral discrimination are discussed. We also investigated the effect of various alcohols as the mobile phase modifiers for the chiral discrimination of 14 racemic glycerin selenium compounds. In recent years, a growing interest has been paid to organic selenium and sulfur compounds for their importance in the life science field. Because stereoisomers often show different biological activities, it is apparently significant work to separate the racemic glycerin selenium and sulfur compounds for the further study of the properties of these chiral compounds.

Experimental

Chemicals

Analytical-grade ethanol, 1-propanol, 2-propanol, 1-butanol, *tert*-butanol, and *n*-hexane were purchased from Tian-Jin Second Chemicals Factory (Tian-Jin, China). Spherical silica gel (5-µm mean particle size, 13-nm mean pore size, and a specific surface area of 110 m^2/g) was prepared in-house at our laboratory. CDMPC, cellulose tris-phenylcarbamate (CTPC), and cellulose tris-4-methylbenzoate (CTMB) were synthesized as described in previous studies (4,5), with small modifications.

Chromatography

The chromatographic system was combined with a Shimadzu (Kyoto, Japan) LC-6A system including a solvent delivery pump, a Model SPD-6AV variable-wavelength detector (Shimadzu), and a Rheodyne Model 7125 valve injector equipped with a 20-µL loop and a Model C-R3A chromatopac processor (Shimadzu).

The flow rate of the eluent was kept at 0.5 mL/min throughout the experiments and the detection wavelength was kept at 254 nm. All experiments were carried out at room temperature. The

major component of the mobile phase was *n*-hexane. Different structures of alcohol (such as ethanol, 1-propanol, 2-propanol, 1-butanol, and *tert*-butanol) were used as a mobile phase modifier. All mobile phases were filtered through a 0.45-µm filter and degassed prior to use.

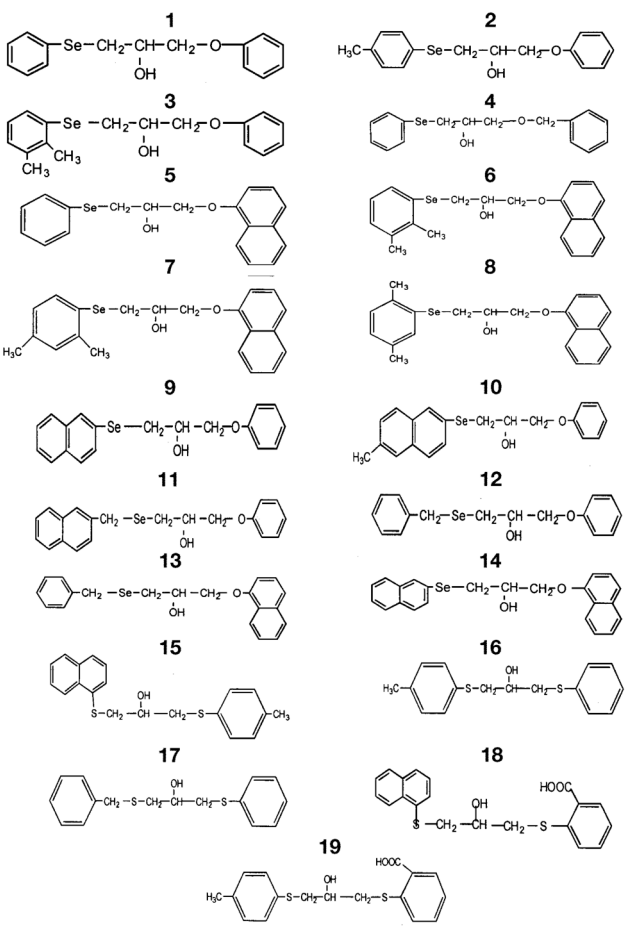

Figure 1. Structures of the racemic compounds 1–19.

CSPs

CDMPC was prepared by the method of a previous study (4). The CSP used was prepared by dissolving 0.52 g CDMPC in 30 mL solvent (THF). Ten milliliters of the CDMPC–THF solution was added to 3.0 g silica gel pretreated with 3-aminopropyltriethoxysilane, the mixture was stirred, and then the solvent was removed under reduced pressure. This coating process was repeated three times. The CDMPC coating amount reached approximately 15% (w/w). Using the same procedure, the CTPC CSP and CTMB CSP were prepared. The chiral packing materials were respectively packed by the slurry method into a 150- × 4.6-mm-i.d. stainless steel column at a high pressure of 5.88×10^7 Pa.

Racemic compounds

Dr. Yu-Lai Hu (Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, China) kindly provided racemic compounds 1 through 19 (the chemical structures of these compounds are shown in Figure 1). These compounds were synthesized in order to develop new drugs. We believed that some of them were the first direct chiral separations on cellulose-based CSPs.

Results and Discussion

Satisfactory resolutions on cellulose-based CSPs were obtained under different conditions for all analytes, except for analytes 5, 6, and 16. The chromatographic results for the chiral separation of racemates 1–14 are summarized in Table I. The chromatographic results of the chiral separation of racemates 15–19 are summa-

Table I. Chromatographic Results of the Chiral Separation of Racemates 1–14*

	n-Hexane–ethanol (80:20, v/v)			*n*-Hexane–1-propanol (80:20, v/v)			*n*-Hexane–2-propanol (80:20, v/v)			*n*-Hexane–1-butanol (80:20, v/v)			*n*-Hexane–*t*-butanol (80:20, v/v)		
	k_1†	α‡	Rs§	k_1	α	Rs	k_1	α	Rs	k_1	α	Rs	k_1	α	Rs
1	1.56	1.23	1.10	1.92	1.45	1.14	2.41	1.49	2.16	2.03	1.56	2.33	3.28	1.81	4.08
2	1.50	1.00	0.00	1.88	1.30	0.96	2.46	1.47	2.38	1.86	1.41	1.57	3.88	1.59	3.42
3	1.66	1.40	1.20	1.75	1.80	1.08	1.42	1.84	1.45	2.17	1.97	2.35	4.16	2.00	2.61
4	1.62	1.21	0.95	2.33	1.34	1.45	2.90	1.38	2.05	2.47	1.41	2.08	3.95	1.57	3.20
5	4.67	1.00	0.00	7.13	1.00	0.00	4.64	1.00	0.00	7.75	1.00	0.00	14.74	1.00	0.00
6	4.33	1.00	0.00	6.94	1.00	0.00	4.64	1.00	0.00	7.66	1.00	0.00	13.4	1.08	0.13
7	3.57	1.00	0.00	5.72	1.00	0.00	6.51	1.00	0.00	6.25	1.00	0.00	9.63	1.12	0.82
8	1.62	1.39	1.13	1.61	1.71	1.12	1.39	1.70	1.38	2.00	1.80	1.79	4.24	2.05	2.80
9	3.40	1.24	0.75	3.57	1.40	0.82	3.79	1.35	1.38	4.72	1.46	1.50	10.6	1.43	1.86
10	1.76	1.39	0.75	1.66	1.73	1.41	1.57	1.66	1.10	2.11	1.82	2.40	4.86	1.96	3.28
11	4.14	1.66	2.27	4.87	2.07	2.52	4.54	2.44	3.70	4.47	2.55	3.85	11.8	1.47	1.04
12	2.37	1.25	1.25	2.47	1.46	0.95	2.21	1.42	0.72	3.49	1.57	2.23	6.27	1.59	2.09
13	4.50	1.00	0.00	7.37	1.20	1.21	7.05	1.12	0.68	5.48	1.12	0.15	13.6	1.07	0.25
14	7.75	1.00	0.00	11.9	1.11	0.67	14.0	1.00	0.00	11.6	1.14	0.78	22.0	1.22	1.25

* 0.5-mL/min flow rate, 254-nm detection wavelength, and 0.02 AUF.
† k_1, retention factor.
‡ α, stereoselectivity.
§ Rs, resolution factor.

rized in Table II. In Figure 2 the resolution of racemates 10, 11, 12, 15, and 19 are shown as several typical examples of the separations achieved on the CDMPC CSP.

Chiral separation of the racemic glycerin selenium and glycerin sulfur compounds

Mixtures of *n*-hexane with different alcohols as mobile phases were tested to optimize the chiral resolution of racemates 1–14 on the CDMPC CSP. The increase of the chain length of the alcohol (from ethanol and 1-propanol to 1-butanol) did not have a dramatic effect on the retention factor and stereoselectivity of the racemates except for 13 and 14. When *t*-butanol was used instead of 1-butanol or when 2-propanol was used instead of 1-propanol to increase the steric bulk of the mobile phase modifier, the retention factor and stereoselectivity of the analytes did not change distinctly except for 13 and 14. For compounds 13 and 14, partial resolution or total loss of resolution was observed using 2-propanol instead of 1-propanol as the modifier in the mobile phase. This result indicated that the mobile phase modifier, alcohol, competed with the analyte for the hydrogen-bonding sites on the CSP, but the chain length and steric bulk of alcohol as the mobile phase modifier played a less important role on chiral separation. The primary factor of chiral recognition generally depends on the structure of the solute.

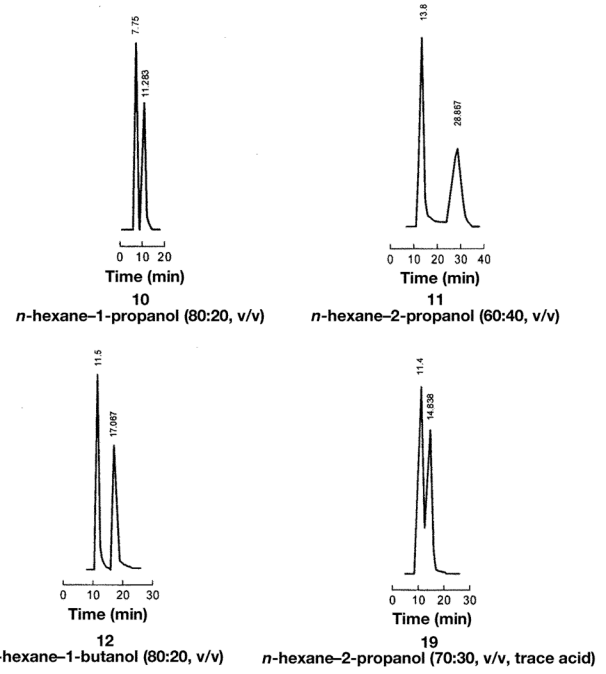

Figure 2. Chromatograms of compounds 10, 11, 12, and 19 (0.5-mL/min flow rate, 254 nm, 0.02 AUFS).

We selected *n*-hexane–2-propanol as the mobile phase for the separation of the racemic glycerin sulfur compounds 15–19 on the CDMPC CSP. For compounds 18 and 19 (which contained the carboxylic acid group) a small amount of trifluoroacetic acid was added to the mobile phases in order to facilitate elution. Optimization of the resolution for compounds 18 and 19 was obtained by increasing the amount of 2-propanol in the mobile phase. Compounds 15 and 16 obtained separation in *n*-hexane–*t*-butanol (90:10, v/v), and compound 17 obtained slight separation in *n*-hexane–2-propanol (90:10, v/v).

Effect of the structure of the solute on chiral separation

All of the glycerin selenium compounds and glycerin sulfur compounds examined contained hydroxyl groups that could interact with the carbamate group of the CSP by hydrogen bonding, aromatic groups that could interact with the phenyl groups of the CSP by charge-transfer (π–π) interaction, and the steric hindrance effect.

According to the results in Table I, the chiral resolution of compounds 1–14 to a certain extent can be improved by changing the type and strength of the alcohol modifier. This shows that the competition with the CSP to form hydrogen bonds between the solute and modifier may be a key factor for chiral resolution. By comparing all of the compounds that had the phenyl group (solutes 1, 2, 3, 4, and 12) with all compounds having the naphthyl group (solutes 5, 6, 7, 8, 9, 10, 13, and 14), we found that the glycerin selenium compounds having the phenyl group were better resolved and had a shorter retention than the compounds having the naphthyl group. Compound 14 (which contains two naphthyl groups in the molecule) gave the strongest retention under the same chromatographic conditions on the CDMPC CSP. Apparently, the naphthyl group in the molecule provided stronger π–π charge-transfer interaction and greater steric hindrance than the phenyl group. However, this π–π charge-transfer

Table II. Chromatographic Results of the Chiral Separation of Racemates 15–19*

	n-Hexane–2-propanol												*n*-Hexane–*t*-butanol		
	60:40 (v/v)			70:30 (v/v)			80:20 (v/v)			90:10 (v/v)			90:10 (v/v)		
	k_1†	α‡	Rs§	k_1	α	Rs	k_1	α	Rs	k_1	α	Rs	k_1	α	Rs
15	1.62	1.15	0.20	2.24	1.15	0.31	2.54	1.20	0.72	7.84	1.21	0.95	9.33	1.23	1.00
16	0.98	1.00	0.00	1.32	1.11	0.13	1.45	1.14	0.35	4.40	1.15	0.64	5.12	1.16	0.71
17	1.07	1.00	0.00	1.64	1.00	0.00	1.41	1.00	0.00	4.91	1.07	0.24	6.33	1.00	0.00
18	3.43	1.38	0.31	5.88	1.39	0.78	5.51	1.50	1.17						
19	1.73	1.44	0.69	3.26	1.37	0.85	3.01	1.51	1.29						

* 0.5-mL/min flow rate, 254-nm detection wavelength, and 0.02 AUF.
† k_1, retention factor.
‡ α, stereoselectivity.
§ Rs, resolution factor.

interaction from the naphthyl group was so strong that a negative effect may have been induced for chiral recognition.

By comparing the relationship between structure and chiral recognition in detail for compounds 1, 2, 3, 4, and 12, the addition of the methyl group on the phenyl ring or the introduction of methylene between the ether oxygen (or selenium) atom and the phenyl ring resulted in a decrease of the resolution for compounds 2, 3, 4, and 12. Although, the retention factor and the stereoselectivity increased for some of them. More specifically, repulsive electron methyl or methylene on phenyl groups enhanced the π–π charge-transfer interaction between the solutes (2, 3, 4, and 12) and the CSP, but the steric hindrance of them contributed a more obvious negative role for chiral recognition. However, contrary to compounds 2, 3, 4, and 12, when the methyl group or methylene group were introduced to the naphthyl group for the compounds with naphthyl groups (5–11, 13, and 14), there was a positive effect for the chiral discrimination because of the steric hindrance that weakened the too strong π–π charge-transfer interaction between the naphthyl group and the CSP. As can be deduced from the data in Table I, compound 5 is the most difficult to resolve under these same conditions. We can also carry out a comparison for the retention and resolution between compound 9 and compounds 10 and 11. They differ from 9 only in the addition of a methyl group on the naphthyl ring or a methylene group between the naphthyl group and the selenium atom. The results showed that 10 and 11 obtained better separation than 9. Apparently, the naphthyl group provided a stronger π–π charge-transfer interaction and steric hindrance than the phenyl group. Also, the π–π charge-transfer interaction between the aromatic group on the analyte and the phenyl moieties on the CSP was an effect not to be ignored for the chromatographic retention and resolution. We also found that the addition of the methyl group between the aromatic group and selenium resulted in a greater electronic effect and a weaker steric effect (caused by the aromatic group) than those without the methyl group. The solutes with this structural feature (11 and 12) obtained better separation than solutes 1, 2, 3, 4, 9, and 10 using ethanol as the mobile phase modifier.

When the phenyl ring linked to the ether oxygen atom in the molecule was replaced by the naphthyl ring (which can be observed through the analytes between compounds 1, 3, 9, and 12 and compounds 5, 6, 13, and 14), several different chiral resolution results were obtained. The compounds 1, 3, 9, and 12 can more easily be resolved than 5, 6, 13, and 14 under the same chromatographic conditions. From these results, we deduced that the steric hindrance of the naphthyl group that was linked to the ether oxygen atom of the analyte contributes a negative effect for chiral resolution.

Another interesting phenomenon was observed resulting from the subtle discrepancy of the structure in the chiral compounds that cause different chiral resolutions. In regards to compounds 6, 7, and 8, they differed only in the addition of the methyl group in the *o*- and *m*-position or the *o*- and *p*-position on the phenyl ring linked to the selenium atom in their molecules, respectively. The difference between 6, 7, and 8 was mainly their steric hindrance. Compound 8 obtained the best chiral separation for all of the mobile phases. Compound 7 obtained separation only using *tert*-butanol as the modifier, but compound 6 could not be separated under all conditions.

We analyzed the glycerin sulfur compounds using the same method as for the glycerin selenium compounds. For solutes 18 and 19, the hydrogen-bonding interaction between the -COOH group of the analyte and the carbamate residues of the CSP may have played a strong role in the chiral discrimination process. This interaction is so strong that CF_3COOH has to be added into the mobile phase to suppress it in order to obtain chiral separation. It is noteworthy to compare compounds 15, 16, and 17 with the previously mentioned glycerin selenium compounds. The main difference is that they do not have the ether oxygen atom that may provide the additional hydrogen-bonding interaction for the chiral discrimination process. The resolutions of solutes 16 and 17 are far more inferior to that of the corresponding compounds 12 and 2.

Enantioseparation of 15–19 on the CDMPC, CTPC, and CTMB CSP

In order to explain the mechanism of chiral recognition better, we compared the retention and stereoselectivity of glycerin sulfur compounds on the CDMPC-, CTPC-, and CTMB-CSP columns respectively under identical conditions. The results that were obtained are given in Table III, showing significant differences with respect to the retention time, stereoselectivity, and resolution factor. The usefulness of operating three columns for the separation of the glycerin sulfur enantiomers was the observation of

Table III. Results of the Separations of the Glycerin Sulfur Racemates*

	CDMPC CSP				CTMB CSP				CTPC CSP			
	k_1†	k_2‡	α§	Rs**	k_1	k_2	α	Rs	k_1	k_2	α	Rs
15	2.54	3.04	1.20	0.72	6.25	7.50	1.20	0.40	1.53	–	1.00	0.00
16	1.45	1.66	1.14	0.35	3.08	–	1.00	0.00	1.11	–	1.00	0.00
17	1.41	–	1.00	0.00	4.44	–	1.00	0.00	1.35	–	1.00	0.00
18	5.51	8.27	1.50	1.17	17.5	–	1.00	0.00	6.29	7.68	1.22	0.78
19	3.01	4.68	1.51	1.29	5.90	7.60	1.29	0.55	3.79	4.69	1.24	0.89

* *n*-Hexane–2-propanol mobile phase (80:20, v/v, trace CF_3COOH); 0.5-mL/min flow rate; 254-nm detection wavelength; and 0.02 AUFS.
† k_1, retention factor of the first elution enantiomer.
‡ k_2, retention factor of the second elution enantiomer.
§ α, stereoselectivity.
** Rs, resolution factor.

different results. The CDMPC CSP had the highest resolving power between all the columns investigated.

The molecular recognition mechanism was assumed to involve the formation of π–π interactions between the benzyl group of the selector and the aromatic ring of the analyte, hydrogen bonding between the analyte and CSP, and steric interactions. It was obvious from our results that the individual contributions differed significantly for the investigated CSPs.

Comparing the data obtained from the CTPC CSP and CDMPC CSP (phenylcarbamate versus dimethylphenylcarbamate) illustrated the importance of hydrogen-bonding interaction between the carbonyl oxygen of analytes 18 and 19 and the NH of the cellulose phenylcarbamate as well as between the OH proton of the analyte and the carbonyl oxygen of the CSP. Compounds 15, 16, and 17 without -COOH groups were not separated on the CTPC column, but compounds 15, 16, 18, and 19 were separated on the CDMPC CSPs.

Comparing the CDMPC CSP with the CTMB CSP led to suggestions about the contribution of the binding group and the distance of the phenyl group from the stereo center. A significantly higher retention factor was obtained with CTMB than with CDMPC. This may be because of the strong hydrogen bonding between the carbonyl oxygen of the CTMB and the OH proton of the analyte. The stereoselectivity of CTMB decreased because the distance decreased between the asymmetric center of the stationary phase and that of the glycerin sulfur racemates. When comparing the stereoselectivity of various racemic glycerin sulfurs on CTMB, the importance of steric and π–π interactions became evident. There were more steric hindrances on the naphthyl group and stronger π–π interactions in racemate 15 than in racemates 16 and 17 with the phenyl group. Compound 15 obtained separation, and also compound 18 had a naphthyl group in the molecule, but compound 18 failed to separate because of the additional hydrogen-bonding interaction between the -COOH group of the analyte and the CTMB CSP. When comparing compounds 16 and 19, additional hydrogen bonding enabled compound 19 to be separated.

These results indicated that for the substituent groups on the nonchiral atom, there could take place a direct or indirect effect for chiral discrimination, and both the π–π and steric interactions played an opposite role with the hydrogen-bonding interactions in the chiral discrimination process.

Conclusion

All of the compounds studied in this work were involved with the hydrogen-bonding interaction and π–π charge-transfer interaction because there were aromatic groups and hydroxyl groups in the structure of their molecules. According to the results of this investigation, we can make some conclusions for the chiral discrimination mechanism involved in the glycerin selenium and sulfur racemates. The first conclusion is that all of the enantiomers of the glycerin selenium compounds containing the phenoxy or phenyl methoxyl group were well-resolved, and so were all of the enantiomers in the glycerin sulfur compounds containing the carboxy or naphthyl group. Some of the enantiomers of the glycerin selenium compounds containing the naphthoxy group could be well-resolved; however, other glycerin selenium compounds containing the naphthoxy group and glycerin sulfur compounds obtained no resolution or only partial or slight resolution. The second conclusion is that both of the hydrogen-bonding and π–π charge-transfer interactions could be contributed to the chiral discrimination of the analytes. The hydrogen-bonding interaction between the hydroxyl groups on the chiral atom in the molecule and the carbamate residues of the CSP play a key role for chiral discrimination. We can largely change the enantioselectivity of chiral separation by altering the type and the strength of the modifier in the mobile phase. The substituent groups on the nonchiral atom show direct or indirect effect during chiral discrimination. The π–π charge-transfer interactions on the nonchiral atom play an important role for the retention of the analyte on the CSP. The stronger the π–π charge-transfer interactions, then the longer is the retention of the analyte. The third conclusion is that the competition between the hydrogen-bonding and π–π interaction exists in compounds containing both the hydroxyl and aromatic group. The two interactions may be playing an opposite role in the chiral discrimination process for some special racemates. Too strong of an interaction on the nonchiral atom (π–π charge-transfer or hydrogen-bonding interactions) may show a negative effect for the chiral recognition process under normal chromatographic condition. The final conclusion is that the steric hindrance is not ignored for chiral recognition. The subtle discrepancy of the structure in the molecule may change the enantioselectivity of the separation. Although this variation is far from the chiral center, it is important for the effect of steric hindrance.

Acknowledgments

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