Comparison of Two High-Pressure Liquid Chromatographic Assays for Carboprost, a Synthetic Prostaglandin

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Abstract □ A high-pressure liquid chromatographic assay for carboprost tromethamine as the bulk drug and in a sterile solution formulation is described. The procedure involves derivatization of the prostaglandin to form the UV-absorbing naphthacyl ester, which then is chromatographed on a silica gel column using methylene chloride–1,3-butanediol–water (496:4:0.25) as the mobile phase. This procedure is compared with a nonderivatization procedure with refractive index detection. Both procedures separate the 15*R*-epimer of carboprost from carboprost, but only the derivatization procedure separates the 5-*trans*-isomer of carboprost. Possible reasons for the better separation using the derivatization procedure are discussed. Both procedures gave a coefficient of variation of ~1% for carboprost. The derivatization procedure gave a coefficient of variation of ~7% for the 15*R*-epimer and 5-*trans*-isomer when present at 2% of the carboprost level.

Keyphrases □ Carboprost—comparison of high-pressure liquid chromatographic assays with and without derivatization □ High-pressure liquid chromatography—analysis, carboprost, comparison of procedures with and without derivatization □ Prostaglandins—carboprost, high-pressure liquid chromatographic analysis, comparison of procedures with and without derivatization

Several reviews of prostaglandins have been published. Oesterling *et al.* (1) reviewed the nomenclature, chemistry, stability, analytical methods, metabolism, and biology of many prostaglandins. Analytical methods have been developed for the assay of prostaglandins in biological matrixes using GLC, radioimmunoassay, and GLC–mass spectrometry. Liquid chromatography has been used to separate prostaglandins of similar structures (2–4).

The high-pressure liquid chromatographic (HPLC) assays presented here were developed for carboprost [(15*S*)-15-methyldinoprost, I] and its possible impurities or degradation products in bulk drug and pharmaceutical formulations. A similar prostaglandin, dinoprost (II), was used as the internal standard in the nonderivatization procedure.

Carboprost and dinoprost are present initially as their tromethamine salts. The free acids are formed during extraction.

EXPERIMENTAL

Nonderivatization Assay—*HPLC Conditions*—A high-pressure liquid chromatograph[1] was used with a differential refractometer detector[2]. Two 3.9-mm × 30-cm microparticulate silica gel columns[3] were used in series. The flow rate of the mobile phase was maintained at 1 ml/min, and a 25-μl injection volume was used with a loop injector[4]. The chart speed was 0.64 cm/min, and attenuation of the refractive index detector was set at 9.6×10^{-5} unit full scale. The mobile phase was acetonitrile–methanol–acetic acid (96:3:1).

Internal Standard Solution—An aqueous solution containing ~3.4 mg of dinoprost tromethamine/ml was prepared.

Bulk Drug Carboprost Tromethamine Reference Preparation—To a separator were added ~3.3 mg of carboprost tromethamine reference standard, accurately weighed, 1.0 ml of the internal standard solution, and 0.5 g of boric acid. The mixture was swirled to dissolve the boric acid partially, and the carboprost free acid and the internal standard were extracted from the aqueous layer using two 5-ml portions of chloroform. The chloroform extracts were combined and evaporated to dryness with a nitrogen stream.

Carboprost Tromethamine Sterile Solution Reference Preparation—Ten milliliters of 0.9% benzyl alcohol[5] in water was added to a separator, followed by the same additions as used for the reference preparation of bulk drug carboprost tromethamine.

Bulk Drug Sample Preparation—The same procedure as that for the reference preparation was used, substituting the bulk drug sample for the carboprost tromethamine reference.

Carboprost Tromethamine Sterile Solution Sample Preparation—Ten milliliters of sterile solution (0.25 mg carboprost equivalents/ml) was added to a separator, followed by 0.5 g of boric acid and 1.0 ml of the internal standard solution. Carboprost and the internal standard were extracted from the aqueous layer using two 5-ml portions of chloroform. The extracts were combined and evaporated to dryness with a nitrogen stream.

Procedure—To the residues of both the reference and the sample was added 0.5 ml of the mobile phase. The vials were swirled to dissolve the residue and chromatographed using the described HPLC conditions.

Derivatization Assay—*HPLC Conditions*—A high-pressure liquid chromatograph[1] was used with a UV detector[6] at 254 nm. A single 3.9-mm × 30-cm microparticulate silica gel column[3] was used. The flow rate was maintained at ~1.8 ml/min, and a 10-μl injection volume was used with a loop injector[4]. The recorder chart speed was 0.64 cm/min, and attenuation on the UV detector was set at 0.08 aufs. The mobile phase was methylene chloride–1,3-butanediol–water (496:3.5:0.25).

Internal Standard Solution—A mobile phase solution containing ~3 or 7 mg of guaifenesin/ml was prepared for the assay of carboprost sterile solution (0.25 mg/ml) or the bulk drug, respectively.

Derivatization Reagents—An acetonitrile solution containing 20 mg of α-bromo-2-acetonaphthone[7] or 10 μl of diisopropylethylamine[8]/ml was used.

Bulk Drug Reference Preparation—Approximately 5.0 mg of carboprost tromethamine reference was weighed accurately and transferred to a stoppered 35-ml centrifuge tube.

Sterile Solution Reference Preparation—A solution containing ~0.332 mg of carboprost tromethamine reference/ml in water and 9 mg of benzyl alcohol[5]/ml was accurately prepared.

Bulk Drug Sample Preparation—Approximately 5 mg of the carboprost tromethamine sample was weighed accurately and transferred to a 35-ml centrifuge tube.

Sterile Solution Sample Preparation—The solution (0.25 mg of carboprost equivalent/ml) was used without dilution.

Procedure—For bulk drug, 20.0 ml of methylene chloride and 2.0 ml of citrate buffer (0.5 *M*, pH 4.0) were added to the sample and reference tubes. After the tubes were shaken for 10 min and centrifuged, the top aqueous layer was discarded. A 4.0-ml aliquot of the methylene chloride layer was transferred to a small vial and evaporated to dryness. Eighty microliters of the α-bromo-2-acetonaphthone solution was added, followed by 60 μl of the diisopropylethylamine solution. The vials were swirled to dissolve the residue and allowed to stand for 1 hr or heated at 30–35° for 15 min. Then the solutions were evaporated to dryness, and 2.0 ml of the internal standard was added. After mixing, the solutions

[1] DuPont model 830.
[2] Waters model R-401.
[3] Waters Micro-Porasil.
[4] Valco.
[5] The benzyl alcohol was added to provide similar extraction conditions for the reference and samples.
[6] Component of DuPont model 830 liquid chromatograph.
[7] Aldrich Chemical Co. The derivation effectiveness of this reagent varied from lot to lot. A satisfactory reagent was obtained by hot filtration through activated charcoal using carbon tetrachloride, followed by recrystallization.
[8] Aldrich Chemical Co.

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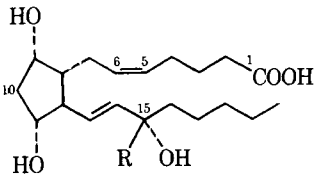

I: R = CH_3
II: R = H

were chromatographed within 24 hr of derivatization using the described HPLC conditions. The standard and sample preparations were protected from light, because light was shown to cause degradation of the naphthacyl ester of carboprost.

For the sterile solution, 2.0 ml of the sample solution and 2.0 ml of the reference solution were added to separate stoppered 35-ml centrifuge tubes. To each tube were added 20.0 ml of methylene chloride and 1.0 ml of citrate buffer. The tubes were shaken for 10 min and centrifuged. The top aqueous layer was discarded, and an 8.0-ml aliquot of the methylene chloride layer was transferred to a small vial and evaporated using a nitrogen stream (complete evaporation was not obtained due to the presence of benzyl alcohol extracted from the aqueous solution). Forty microliters of α-bromo-2-acetonaphthone solution was added, followed by 30 μl of diisopropylethylamine solution. After swirling to mix and dissolve the residue, the solution was allowed to stand for 1 hr or heated at 30–35° for 15 min. The acetonitrile then was evaporated, and 1.0 ml of the internal standard solution was added. After mixing, the solutions were chromatographed within 24 hr of derivatization using the described HPLC conditions. Standard and sample preparations were protected from light, because light was shown to cause degradation of the naphthacyl ester of carboprost.

Table I—Comparison of Extent of Reaction Completeness for Carboprost Tromethamine and Carboprost Free Acid after 1 hr

Sample Weight, mg	Peak Height Ratio	Ratio/ Weight
	Carboprost Tromethamine	
0.2720	0.6961	2.559
0.2720	0.8815	3.241
0.2720	0.7379	2.713
Mean		2.838
CV, %		12.6
	Carboprost Free Acid	
0.1991	0.9455	4.749
0.2079	0.9926	4.774
0.2297	1.0826	4.713
Mean		4.745
CV, %		0.6

Table II—Reaction of Carboprost with α-Bromo-2′-acetonaphthone Solution Containing Diisopropylethylamine at Several Time Intervals

Minutes	Sample Weight, mg	Peak Height Ratio	Ratio/ Weight
25	0.2614	1.1989	4.586
40	0.2614	1.2787	4.892
60	0.2614	1.2945	4.952
81	0.2614	1.2988	4.969
105	0.2614	1.3039	4.988
150	0.2614	1.3132	5.024

Table III—Precision in the Assay of Carboprost Bulk Drug

Sample Weight, mg	Peak Height Ratio	Ratio/ Weight
	Derivatization Procedure	
2.585	1.601	0.619
2.834	1.766	0.623
2.613	1.648	0.631
2.522	1.567	0.621
2.523	1.596	0.633
Average		0.625
CV, %		0.9
	Nonderivatization Procedure	
3.467	1.567	0.452
3.464	1.571	0.454
3.181	1.452	0.457
3.533	1.592	0.451
3.460	1.588	0.459
3.166	1.453	0.459
Average		0.455
CV, %		0.8

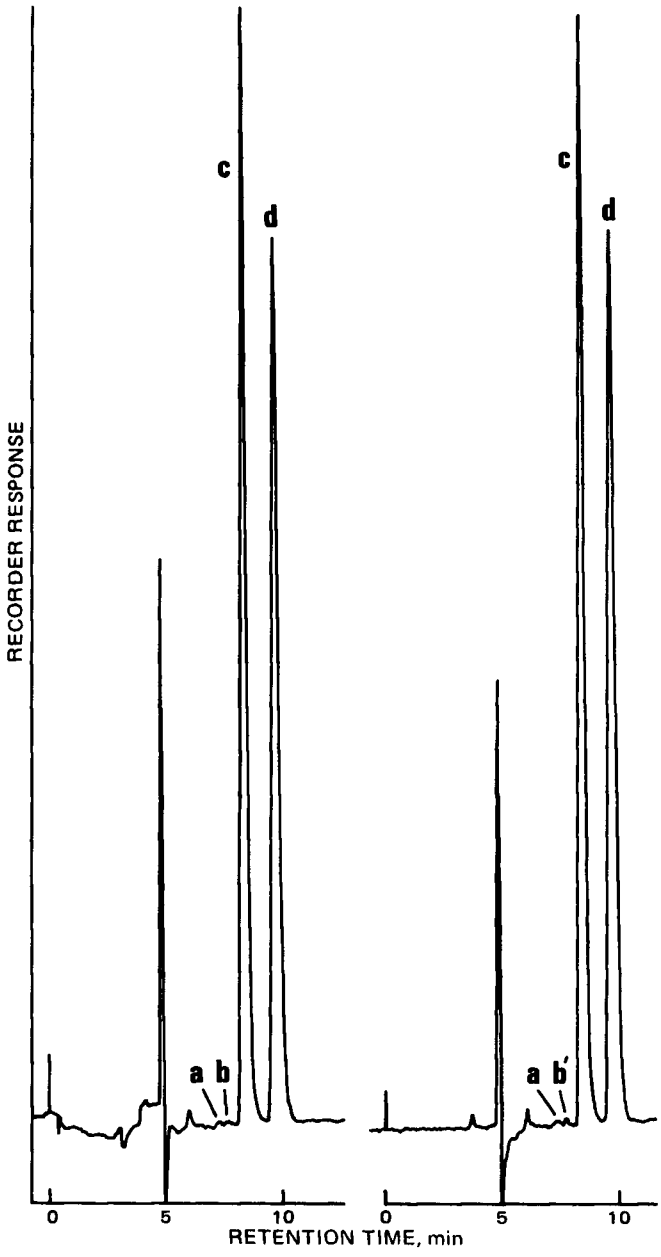

Figure 1—*Chromatograms of the 15R-epimer of dinoprost (a), the 15R-epimer of carboprost at 0.5% (b), the 15R-epimer of carboprost at 1.0% (b′), carboprost (c), and dinoprost (internal standard) (d) using the nonderivatization HPLC procedure with refractive index detection.*

Calculations—For the bulk drug:

$$\% \text{ purity of carboprost tromethamine} = \frac{R_s}{R_{ref}} \times \frac{W_{ref}}{W_s} \times P \quad \text{(Eq. 1)}$$

where R_s is the peak height ratio of the carboprost peak to the internal standard peak in the sample preparation; R_{ref} is the peak height ratio of the carboprost peak to the internal standard peak in the reference preparation; W_{ref} is the weight, in milligrams, of carboprost tromethamine

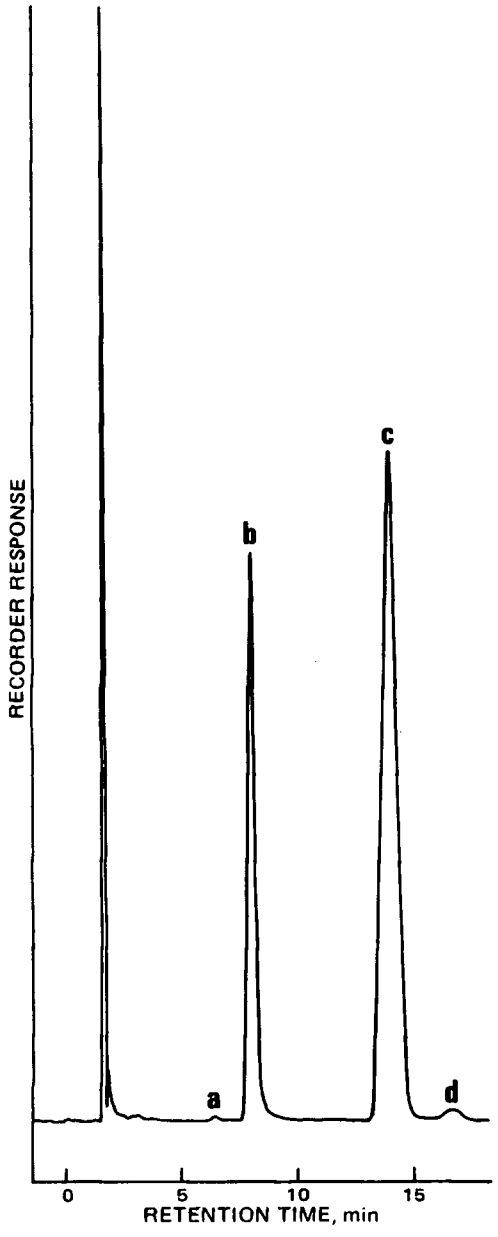

Figure 2—*Chromatogram of guaifenesin impurity (a), guaifenesin (internal standard) (b), 2-naphthacyl carboprost (c), and the 2-naphthacyl 5-*trans*-isomer of carboprost (d) using the derivatization procedure with UV detection.*

amine reference standard; W_s is the weight, in milligrams, of sample; and P is the purity of the carboprost tromethamine reference expressed in percent.

For the sterile solution:

$$\text{mg of carboprost/ml of sterile solution} = \frac{R_s}{R_{\text{ref}}} \times \frac{W_{\text{ref}}}{V_s} \times F_c \times P \qquad \text{(Eq. 2)}$$

where R_s is the peak height ratio of the carboprost peak to the internal standard peak in the sample preparation; R_{ref} is the peak height ratio of the carboprost peak to the internal standard peak in the reference preparation; W_{ref} is the weight, in milligrams, of carboprost tromethamine reference standard used in the procedure; V_s is the volume, in milliliters, of sample; F_c is the factor to convert carboprost tromethamine (mol. wt. 489.65) to carboprost (mol. wt. 368.50) = 0.7526; and P is the purity of the carboprost tromethamine reference expressed as a decimal.

For the impurities:

$$\% \text{ impurity} = \frac{A_a \text{ or } A_c}{A_a + A_b + A_c} \times 100 \qquad \text{(Eq. 3)}$$

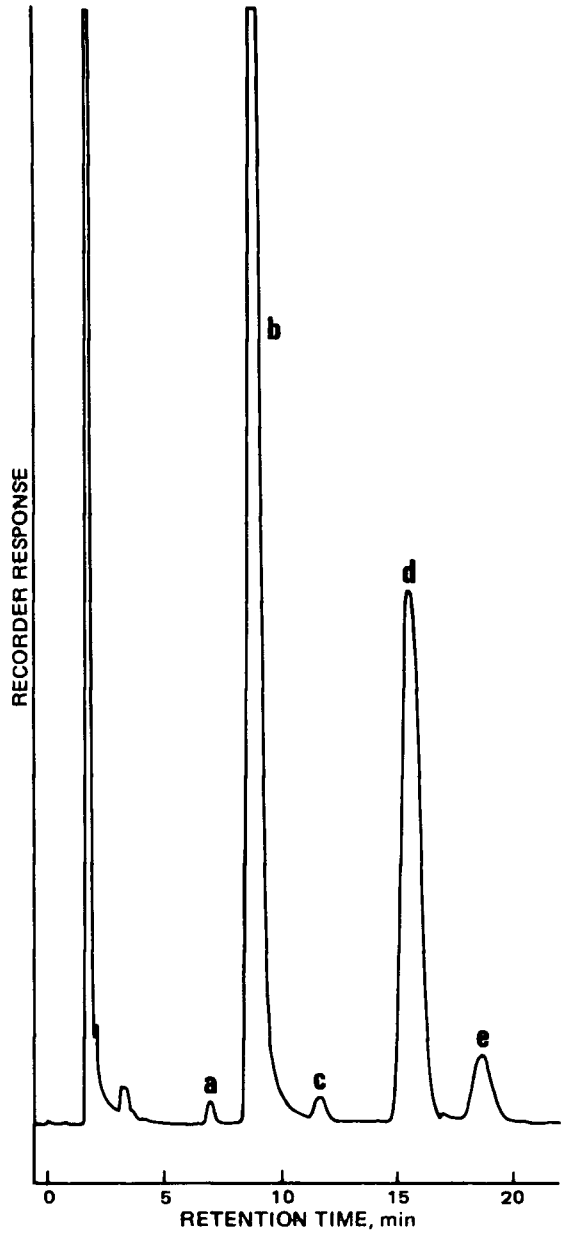

Figure 3—*Chromatogram of a guaifenesin impurity (a), guaifenesin (internal standard) (b), 2-naphthacyl 15*R*-epimer of carboprost at 0.5% (c), 2-naphthacyl carboprost at an attenuation four times that of the other peaks (d), and 2-naphthacyl 5-*trans*-isomer of carboprost at 2.3% (e) using the derivatization procedure with UV detection.*

or:

$$\% \text{ impurity} = \frac{F_aH_a \text{ or } F_cH_c}{F_aH_a + H_b + F_cH_c} \times 100 \qquad \text{(Eq. 4)}$$

where A_a is the area of the 15*R*-epimer peak, A_b is the area of the carboprost peak, A_c is the area of the 5-*trans*-isomer peak, F_a is the factor to normalize the 15*R*-epimer peak height (0.78), F_c is the factor to normalize the 5-*trans*-isomer peak height (1.12), H_a is the peak height of the 15*R*-epimer peak, H_b is the height of the carboprost peak, and H_c is the height of the 5-*trans*-isomer peak.

RESULTS AND DISCUSSION

Both assay procedures (derivatization and nonderivatization) involve extraction of the prostaglandin as the free acid from an acidified aqueous solvent into an organic solvent. This extraction is tailored for the assay of dilute sterile solutions of the prostaglandin in which a concentration step is necessary when using the nonderivatization procedure. Furthermore, the derivatization reaction proceeds much more rapidly with the

Table IV—Assay Results by the Derivatization Procedure of Replicate Samples of the 15*R*-Epimer and 5-*trans*-Isomer in Carboprost Bulk Drug at Concentration Levels of 1.7 and 2.3%

	15*R*-Epimer Content, %	5-*trans*-Isomer Content, %
	1.6	2.4
	1.9	2.5
	1.6	2.6
	1.7	2.4
	1.5	2.3
	1.6	2.0
	1.7	2.4
	1.7	2.4
Average	1.7	2.4
CV, %	7.1	7.4

free acid than with the tromethamine salt (Table I). After 1 hr, the tromethamine salt samples exhibit a low mean ratio and a high variation between samples, while the free acid samples exhibit a high mean ratio and little variation between samples. The derivatization reaction for the free acid is essentially complete after 1 hr (Table II).

Recovery studies showed that the extraction procedure produced nearly complete recovery (~96%) of the prostaglandin. To eliminate possible bias, the reference is carried through the complete procedure along with the sample to be assayed. Replicate samples assayed by either method gave a relative standard deviation of <1% (Table III).

Chromatograms of carboprost (nonderivatization procedure) are shown in Fig. 1; authentic samples of the 15*R*-epimer of carboprost had been added at concentrations of ~0.5 and 1.0% of the carboprost concentration. The epimer peak is baseline separated from the carboprost peak, making the assay satisfactory for stability studies (carboprost degrades to the 15*R*-epimer under acidic conditions). The small peak on the front side of the 15*R*-epimer is the 15*R*-epimer of dinoprost, which was used as the internal standard.

To obtain greater sensitivity for the assay of the 15*R*-epimer in carboprost samples, the derivatization procedure was developed. Formation of the 2-naphthacyl ester has been used for fatty acids (5) and is similar to *p*-nitrophenacyl derivatization of prostaglandins (4). The 2-naphthacyl ester of carboprost was found to exhibit greater absorptivity than the *p*-nitrophenacyl ester at 254 nm with the UV detector. A chromatogram of 2-naphthacyl carboprost using guaifenesin as the internal standard is shown in Fig. 2.

In comparing the nonderivatization and derivatization methods, it is evident that derivatization produced better chromatography. The 15*R*-epimer is better separated, and another impurity, the 5-*trans*-isomer of carboprost, is baseline separated from carboprost. Although the nonderivatization procedure is less time consuming because of the absence of a derivatization step, it is not satisfactory if the 5-*trans*-isomer is present in the carboprost sample. Although several mobile phases were tried, the 5-*trans*-isomer could not be separated from carboprost using the nonderivatization procedure, even with two silica columns in series. With the derivatization procedure, greater separation than is shown in Fig. 2 can be achieved by decreasing the relative concentration of 1,3-butanediol in the mobile phase. This is a definite advantage of the derivatization procedure.

Increased liquid chromatographic separation of the 15-position epimers of carboprost is observed with the derivatization procedure, probably because of the reduced polarity of the carboxyl group by naphthacyl ester formation. The presence of the highly polar carboxyl group masks subtle polarity differences due to the position of the hydroxyl groups in the isomers. Thus, the blocking of the carboxyl group maximizes the effect of the hydroxyl groups, and the polarity differences between the 15-position epimers become more significant.

Figure 3 shows a chromatogram (derivatization procedure) of carboprost with the 15*R*-epimer added. In the absence of reference samples to quantitate the impurities by peak height percent, the 15*R*-epimer and 5-*trans*-isomer peak heights must be multiplied by response factors of 0.78 and 1.12, respectively. The response factors were required to normalize peak height differences due to retention times. These factors were determined using authentic samples. Since the UV chromophore in each compound is the naphthacyl ester, carboprost and the two impurities should have the same absorptivity. By measuring peak area instead of peak height, response factors were not needed. Results by peak area percent showed good agreement with carboprost samples spiked with impurities at known concentrations. Assay results of replicate samples of the 15*R*-epimer at a concentration of 1.7% and of the 5-*trans*-isomer at a concentration of 2.3% in carboprost bulk drug are shown in Table IV. A coefficient of variation of ~7% was obtained for both impurities by peak area percent at 2% of the carboprost concentration.

Light caused degradation of the naphthacyl ester of carboprost. When derivatized samples were placed near a light source, a noticeable increase in the peak corresponding to the 5-*trans*-isomer was observed. No significant change was observed in derivatized samples protected from light for 24 hr. The peak height ratios of carboprost to the internal standard and of the 5-*trans*-isomer to carboprost showed no significant changes. The degradation in light may be due to the chromophore. Acetophenone and several similar compounds have been shown to act as photosensitizers for *cis–trans*-isomerization (6).

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