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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF ACID-CATALYZED DEGRADATION PRODUCTS OF METHYL CARBOPROST IN A POLYMERIC CONTROLLED-RELEASE DEVICE

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SUMMARY

A normal-phase high-performance liquid chromatographic method was used for the determination of methyl carboprost and acid-catalyzed degradation products in a polymer-based, controlled release dosage form. A reversed-phase method was used to isolate sufficient quantities of the degradation products to determine their identity. Degradation of methyl carboprost under acidic conditions results in epimerization and dehydration, to several isomers, at the tertiary allylic hydroxyl group. Mass balance was 94% for a sample allowed to degrade 50%. These compounds were observed to form in the polymer-based, controlled release dosage form. For the determination of methyl carboprost in the dosage form, the method was found to be linear, precise with a relative standard deviation of 2% and to have an average recovery of 99.2%.

INTRODUCTION

Methyl carboprost is the 15(*S*)-15-methyl methyl ester analogue of $PGF_{2\alpha}$ (Fig. 1) and was formulated initially in suppositories[1,2] and later in polymer-based, controlled-release devices. The design of this polymer-based release systems has been previously described[3,4]. Clinical investigation has been in the areas of pregnancy termination, cervical dilation and menses induction. Assay methodology was developed to quantitate the amount of methyl carboprost present in the formulation without interference from other isomers and potential degradation products.

EXPERIMENTAL

Reagents and materials

Reagents were of at least analytical reagent grade quality. Solvents (Burdick

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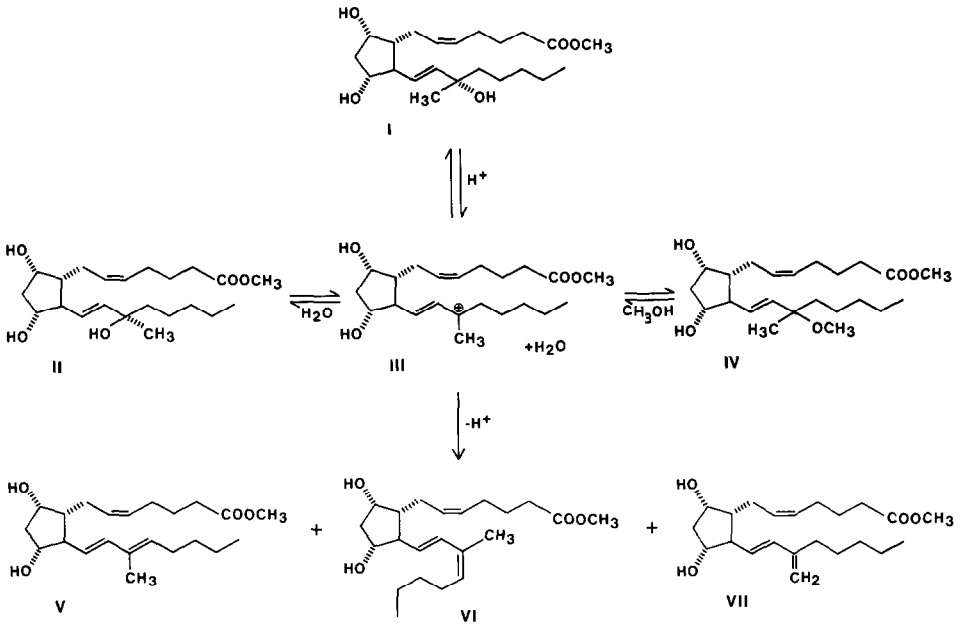

Fig. 1. Predicted pathway of degradation for methyl carboprost: I = methyl carboprost; II = 15(*R*)-epimer; III = carbonium ion intermediate; IV = methoxy derivative (*R* and *S* epimers): V–VI = isomeric dehydration products; VII = methylene derivative.

& Jackson, Muskegon, MI, U.S.A.) used for mobile phases were of distilled-in-glass grade. Methyl carboprost, the 15(*R*)-epimer, the 5,6-*trans* isomer and medroxyprogesterone acetate were available in-house (The Upjohn Company, Kalamazoo, MI, U.S.A.).

Chromatographic instrumentation

A variable-wavelength detector (LDC Model III, Laboratory Data Control, Riveria Beach, FL, U.S.A.), a solvent pump (Altex Model 110, Beckman, Berkeley, CA, U.S.A.) and an autoinjector (WISP Model 710, Waters Assoc., Milford, MA, U.S.A.) were used for the chromatographic analysis.

Chromatographic conditions

The normal-phase system used a 25 cm × 4.6 mm I.D. column packed with cyanopropylsilane bonded to a microparticulate silica (Zorbax cyano, DuPont, Wilmington, DE, U.S.A.) (5–6 μm). The mobile phase was hexane–dioxane–water (750:250:2) at a flow-rate of 2.0 ml/min. The injection volume used was 20 μl. A 3-cm guard column (LiChrosorb CN) was used when samples of the polymer-based, controlled-release device were assayed.

The reversed-phase system used a 25 cm × 4.6 mm I.D. column (analytical separations) or a 25 cm × 9.4 mm I.D. column (semi-preparative-scale system) packed with octadecylsilane bonded to microparticulate silica (Zorbax ODS, DuPont) (5–6 μm). The mobile phase was methanol–water (80:20). For analytical-scale separations, the flow-rate was 1.0 ml/min and the injection volume was 20 μl. For

preparative-scale separations, the flow-rate was 4.0 ml/min and the injection volume was 1.0 ml.

Isolation of degradation products

Degradation products observed using the reversed-phase system were labeled A–E prior to identification. Compounds B, C and E were isolated using the preparative reversed-phase system described above from methyl carboprost (100 mg/ml), dissolved in methanol–0.01 *1M* hydrochloric acid (80:20) and heated at 45°C for 8 h. Compound A was identified as 15(*R*)-methyl carboprost based on identical retention time to an authentic sample. Compound D was not isolated in sufficient purity for identification to be accomplished.

Spectra

The Fourier transform (FT)–IR (Nicolet, Madison, WI, U.S.A.) spectra were obtained from the neat oils on salt plates with a resolution of 8 cm^{-1}. The mass spectra (Varian MAT CH 5-DF, Palo Alto, CA, U.S.A.) of compounds C and E were obtained using electron impact. The probe temperature was 245°C for C and 181°C for E. The ^{13}C NMR (Varian CFT-20, Palo Alto, CA, U.S.A.) spectra were obtained by averaging overnight in C^2HCl_3 solution. The ^1H NMR spectra (Varian CFT-20, Palo Alto, CA, U.S.A.) were obtained at 80 MHz and ambient temperature in C^2HCl_3 solution. Reference IR spectra of 15-methylene and 15-methoxy-$PGF_{2\alpha}$ methyl ester were available in-house.

Assay of polymer-based device

For the normal-phase assay, the membrane (200–400 mg in weight, 10 cm^2 in area, and containing approximately 5–10 mg of methyl carboprost) was removed from the holder and placed in a suitable vial. Internal standard, medroxyprogesterone acetate in mobile phase at 20 μg/ml, was then added in a sufficient amount that the final drug concentration was 1.2 mg/ml. Standard solutions were prepared at the same concentration by dissolving drug in the internal standard solution. Standard and sample preparations were injected into the liquid chromatograph with detection at 214 nm. Approximate retention times of methyl carboprost and medroxyprogesterone acetate were 17 and 12 min, respectively. Peak area measurements were performed with an in-house computer. Ratios of the drug peak area divided by the internal standard peak area were then used to calculate the concentration of drug in samples.

DISCUSSION

The structure of methyl carboprost is shown in Fig. 1 along with several acid-catalyzed degradation products. The most important characteristic of this prostaglandin in regard to degradation is the presence of the tertiary allylic alcohol at C-15. Acid catalyzed formation of a stabilized carbonium ion readily occurs[5]. The stability is due to the delocalization of the charge over a π-bond and a tertiary carbon. For the reverse reaction, replacement of the hydroxyl by water is not stereo-specific and epimerization occurs. Epimerization will continue unless elimination occurs through the loss of a proton from the carbonium ion. This results in the formation

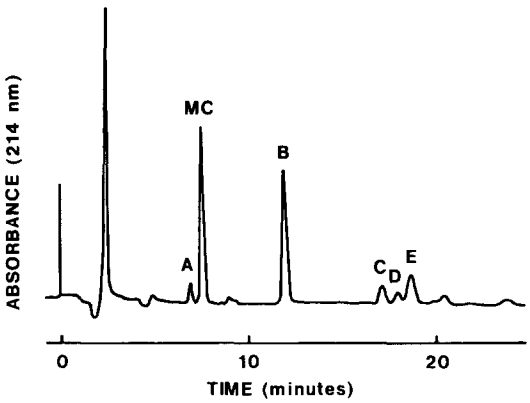

Fig. 2. Chromatogram using reversed-phase conditions after degrading methyl carboprost for 30 min at 45°C in methanol–0.01 *M* hydrochloric acid (80:20). Peaks: MC = methyl carboprost; A = 15(*R*)-epimer; B = 15-methoxy derivative; C,D and E = dehydration products.

of a new double bond with conjugation preferred. The presence of trace acidic substances in formulations could be envisioned to cause degradation to occur. For the development of a stability indicating method, we wanted to insure that the products of acid-catalyzed degradation would be separated from the drug and could be detected to monitor for their formation. As it was not feasible to isolate these products from the formulation, the approach was to degrade the methyl carboprost in an acidic acetonitrile–water solution, isolate and identify the degradation products, then determine if similar products can be found in the degraded formulation.

Two chromatographic methods were used: (i) a previously described normal-phase method using a Zorbax cyano column with a hexane–dioxane–water (750:250:2) mobile phase that is capable of resolving the 15(*R*)-epimer and the 5,6-*trans* isomer from methyl carboprost[6] and (ii) a reversed-phase procedure using a Zorbax ODS column with a methanol–water (80:20) mobile phase which resolves the 15(*R*)-epimer but not the 5,6-*trans* isomer from methyl carboprost. The reversed-phase method provided better resolution of the dehydration products (Fig. 2) than

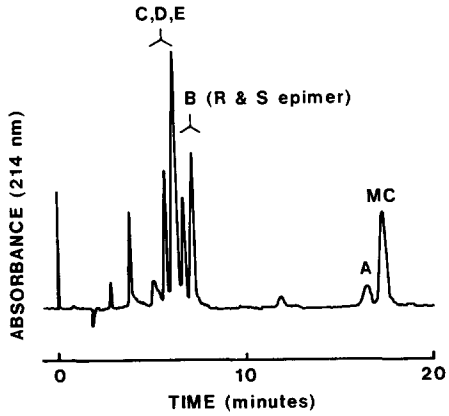

Fig. 3. Chromatogram using normal-phase conditions after degrading methyl carboprost (see legend of Fig. 2 for conditions and labeling identification).

the normal-phase method (Fig. 3). The normal-phase method was advantageous in that the mobile phase caused delamination of the drug containing reservoir from the rate-controlling membrane which results in complete release of the drug in 1 h. The cause of delamination is the swelling of the polymer, to a large extent by the dioxane in the mobile phase. With the reversed-phase procedure, release of the drug could have required several days. The normal-phase chromatography was thus chosen for the analytical method because of the simplicity of the sample preparation and the higher specificity.

Degradation products

Products of acid-catalyzed degradation were generated by heating methyl carboprost at 45°C in a methanol–0.01 *M* hydrochloric acid (80:20). A chromatogram of the degradation products after 30 min under the above conditions is shown in Fig. 2. Identification of the compounds in the peaks was as follows:

Compound A. The retention time of this peak matched that for an authentic sample of the 15(*R*)-epimer of methyl carboprost.

Compound B. The ^{13}C NMR spectrum of this material indicated the presence of only four vinyl carbons, the same number as are present in methyl carboprost. Comparing the ^{1}H NMR spectra of B and the parent meterial (methyl carboprost) indicated that a great deal of structural similarity exists. The most striking difference was the appearance of an additional $-OCH_3$ resonance in B. This evidence is consistent with structure IV in Fig. 1. Further evidence for this structure is that B is not formed when methanol is replaced by acetonitrile in the decomposition solution. The IR spectra also matched that of a reference spectra.

Compound E. The ^{13}C NMR spectrum of compound E indicated the presence of six vinyl carbons in this material, while the mass spectrum indicated the presence of a molecular ion at *m/z* 364. This evidence is consistent with a dehydration product of methyl carboprost.

Comparison of the IR spectrum of this material with spectra of known compounds of this type indicated the basic ring structure in E is the same as the parent material. This means that the hydroxyl groups at C-9 and C-11 are still intact and that no dehydration has occurred in the ring. A sharp band at 960 cm^{-1} which is characteristic of a trans HC–CH in these materials was also observed.

Comparing the NMR spectrum of compound E with the methyl carboprost spectrum indicated the shift of the methyl group on C-15 from 1.2 ppm in the parent to 1.7–1.8 ppm in compound E. In addition to this, the vinyl proton resonances at *ca.* 6.2 ppm are seen in compound E and are not observed in the parent. The large coupling observed (*ca.* 7 Hz) in these vinyl protons (6.2 ppm) is characteristic of *trans* coupling. The chemical shifts of the methyl group and the vinyl protons at *ca.* 6.2 ppm are very similar to those observed in the proton spectrum of isoprene. Based on this evidence the structure of compound E is believed to be V or VI in Fig. 1. Structure VII in Fig. 1 is not compound E because the ^{1}H NMR spectrum obtained on a known sample of VII is clearly different than the ^{1}H NMR spectrum of compound E.

Compound C. The mass spectrum of compound C gives a molecular ion at *m/z* 364 which also is consistent with a dehydration product of methyl carboprost. The IR spectrum observed for this material is nearly indistinguishable from the IR spec-

trum of compound E which again indicates the presence of a trans H–C = C–H and that dehydration did not occur at the C-9 or C-11 ring positions.

The ^{1}H NMR of compound C is also very similar to that of compound E with the major differences being the larger chemical shift (*ca.* 6.5 ppm compared to *ca.* 6.2 ppm) of the vinyl proton in compound C. Since the ^{1}H NMR of compound C does not match the spectrum of a known sample of VII (Fig. 1), compound C is represented by either structure V or VI in Fig. 1. Thus structures V and VI are both possible for compounds C and E. The available data do not allow the unique isomeric assignment of compounds C and E.

Compound D. Adequate material was available for compound D, but because the material could not be isolated pure, no spectra were obtained.

A chromatogram of the degradation products in the normal-phase system is shown in Fig. 3. All the potential degradation products are well resolved from methyl carboprost. Although there are many peaks present, only three contained sizeable quantities of material. Compound D was not isolated without significant contamination from peaks C and E. These compounds appear to be the stable end products of the acidic degradation of methyl carboprost. Peaks C and E were identified as dehydration products. Conjugation was confirmed by their detectability at 254 nm. The exact configuration of these isomers was not determined. The *exo* isomer (VII) was definitely ruled out as one of the two major dehydration products based on the NMR spectra of an authentic compound. The response factors at 214 nm were calculated for compounds C and E. They were found to both have 20 times the absorption of methyl carboprost. Compound B had similar absorption as methyl carboprost as would be predicted.

A degradation profile at pH 2 in acetonitrile–water (50:50) is shown in Fig. 4.

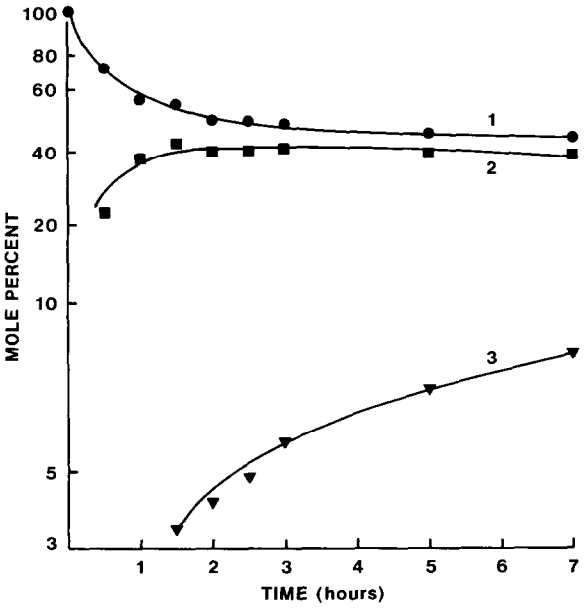

Fig. 4. Degradation profile at pH 2 in acetonitrile–water (50:50). 1 = methyl carboprost; 2 = 15(*R*)-epimer; 3 = sum of dehydration products.

Epimerization occurs rapidly and after 1 h 38% of the drug has been converted to the epimer. As epimerization and dehydration are competing processes, the amount of the 15(*R*)-epimer never equals the amount of the drug. Initial rate of dehydration is approximately 20 times slower than the rate of epimerization. For the degradation profile shown in Fig. 4, 94 ± 1.2% of the drug could be accounted for by summing the 15(*R*)-epimer and dehydration product content of the samples. Approximately five other peaks are usually observed but do not increase in concentration after the first hour. These peaks could account for an additional 3% of the degradation assuming equal response factors to the dehydration products.

Assay for polymer-based device

The polymer based delivery system consisted of a support, a drug-reservoir membrane and a rate-controlling membrane that are laminated together[3,4]. Rate of release can be controlled through varying rate-controlling membrane thickness, diffusional characteristics or the surface area of the device. The membranes were delaminated through use of organic solvents, such as the mobile phase (hexane–dioxane), that cause the polymers to swell. By delaminating the membranes, the drug is extracted reasonably quickly from the device. Aqueous based extraction could have required several days to recover all the drug.

Shaking the membrane in mobile phase for 1 h was required to ensure good recovery and reproducibility. Medroxyprogesterone acetate was found to be a suitable internal standard as it eluted in a time window where polymer related peaks did not occur (Fig. 5). Very little of the polymers were dissolved as evidenced by the amount of residue left upon evaporating a sample preparation.

Recovery was determined by preparing membranes with a known amount of

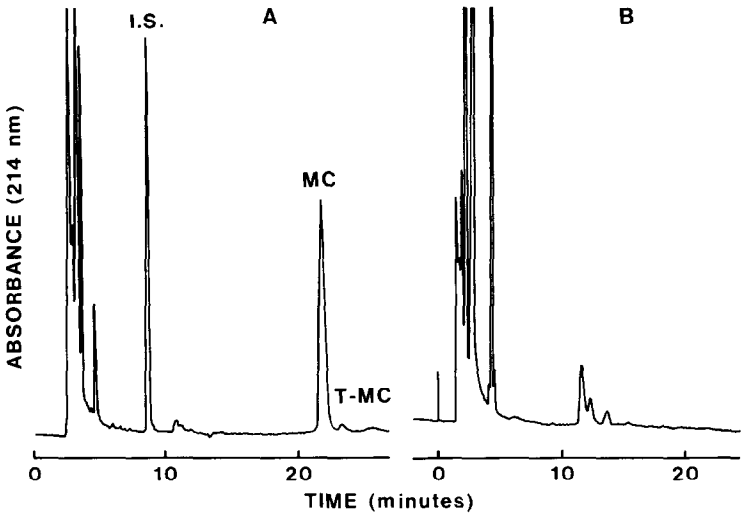

Fig. 5. Chromatograms of analysis of polymer based device using normal-phase conditions. A, Chromatogram of device containing drug; B, chromatogram of device containing no drug showing peaks arising from polymers. Peaks: IS = internal standard (medroxyprogesterone acetate); MC = methyl carboprost; T-MC = 5,6-*trans* isomer of methyl carboprost.

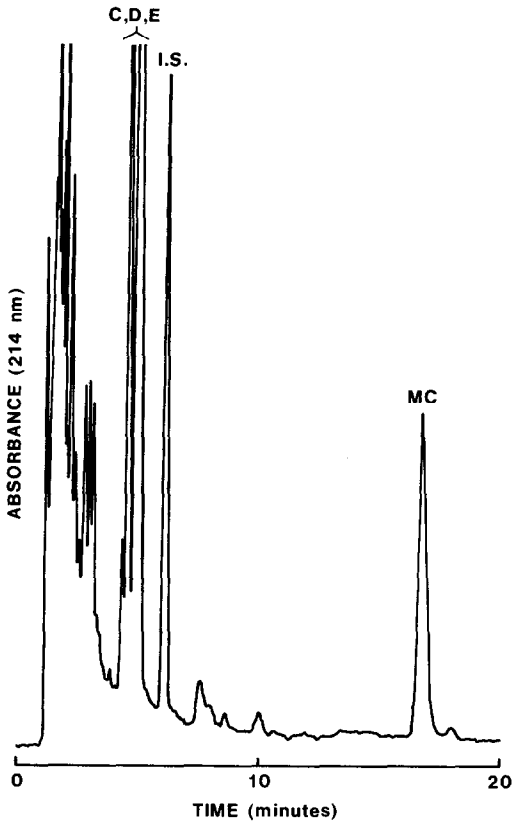

Fig. 6. Chromatogram of a degraded control release device showing the presence of the dehydration products. Peaks: MC = methyl carboprost; IS = internal standard; C,D and E = dehydration products.

drug. The polymers and methyl carboprost could be dissolved in tetrahydrofuran to prepare the drug bearing membrane. As the membranes delaminate during the sample preparation, recovery experiments using the unlaminated membranes was judged suitable. Recovery was linear from 3.75 to 18.75 mg per device (correlation coefficient = 0.9994) and the average recovery was 99.2 ± 2.1% when peak areas were used. With peak heights, low recoveries were determined above 12 mg because of some small changes in peak shape. The analysis of five devices showed that the relative standard deviation of the method was 2.0%. Variation of retention times was observed when the sample preparation was twice as concentrated. Because of the effects of residual materials from the sample preparation on retention time, a guard is recommended. The dehydration products are observed in degraded controlled release devices (Fig. 6). A chromatogram from a controlled release device stored at 47°C for 10 months shows the presence of the dehydration products. All of the degradation products can be seen by the normal-phase assay, but because of their rapid elution, resolution is lost (Fig. 3).

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