derivatization. The resulting solution was cooled to room temperature, and 1.0-μl aliquots were used for subsequent analyses. Reference standards of psilocin and psilocybin were derivatized in the same manner, and quantitation was performed using an external standard of bis(trimethylsilyl)psilocin following the general method described by Gudzinowicz (19). The silanized solutions were stable for several weeks when stored at 4° under anhydrous conditions.

RESULTS AND DISCUSSION

Under the temperature-programmed chromatography conditions described (SE-30), the retention times for bis(trimethylsilyl)psilocin (I) and tris(trimethylsilyl)psilocybin (II) were 8.45 and 13.10 min, respectively. These two compounds were clearly separated from other components in the natural extract. Comparison with the external standard showed the concentrations to be 0.420% psilocin and 0.168% psilocybin. These values are not inconsistent with the total yields obtained by Heim and Hofmann (20) from various cultivated strains of *P. cubensis*. However, in the present analysis, the ratio of psilocin to psilocybin consistently exceeded 1, in contrast to the isolated yields of each compound reported by Heim and Hofmann (20). The present results were confirmed by TLC. The difference in these results might be attributed to the decomposition of psilocin during isolation procedures.

While the mass spectrum of I both as the standard and from the natural extract could be recorded easily, the mass spectrum of II could not be obtained with the longer SE-30 column. However, the conditions utilized with the OV-101 column proved rewarding, and a satisfactory spectrum was recorded. Under these conditions, II was eluted in 3.6 min.

The mass spectrum of I exhibited peaks at *m/e* 348 (M^+) (relative intensity 4%), 333 (1), 291 (6), 290 (21), 75 (3), 73 (39), and 58 (100). The mass spectrum of II showed peaks at *m/e* 500 (M^+) (1%), 485 (1), 442 (6), 77 (9), 75 (15), 73 (47), and 58 (100). While these two spectra are relatively simple in appearance, they are consistent with known fragmentation patterns of this type of compound. The parent peak at *m/e* 348 in I indicates the presence of two trimethylsilyl groups. As expected for 3-indolylethylamines, the significant fragments at *m/e* 290 and *m/e* 58 arise by fission of the bond beta to the nitrogen in the dimethylaminoethyl side chain (21). The M − 15 peak at *m/e* 333 and the peaks at *m/e* 75 and 73 are consistent with known fragmentations of trimethylsilyl ethers (22). The parent peak in the spectrum of II at *m/e* 500, the β-bond fission fragment at *m/e* 442 (M − 58), and the M − 15 peak at *m/e* 485 are evidence for the presence of three trimethylsilyl groups. Again, the peak at *m/e* 58 due to β-bond fission is the most prominent. The mass spectrum of the eluted psilocybin derivative confirms the stability of the silanized molecule under the instrumental conditions used.

Analyses by this method are routinely performed using 5–20 mg of dried fungal tissue.

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 14, 1976, from *148-B West Dana St., Mountain View, CA 94041.*

Accepted for publication July 7, 1976.

The authors thank Dr. Gastón Guzmán for identification and Jonathan Ott for deposition of the specimens at ENCB. They also thank Dr. R. E. Willette, National Institute on Drug Abuse, for supplying standards of psilocin and psilocybin.

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High-Pressure Liquid Chromatographic Separation of 5,6-*cis*- and *trans*-Prostaglandin A_2

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Abstract □ A high-pressure liquid chromatographic procedure capable of separating the *cis*- and *trans*-isomers of prostaglandin A_2 is described.

Keyphrases □ Prostaglandin A_2—high-pressure liquid chromatographic analysis, *cis*- and *trans*-isomers □ High-pressure liquid chromatography—analysis, *cis*- and *trans*-prostaglandin A_2

Prostaglandin A_2 (I) normally has the *cis*-configuration at the 5,6-double bond. However, extracts from the gorgonian *Plexaura homomalla*, a soft coral found in the Caribbean area, contained significant amounts of 5,6-*trans*-prostaglandin A_2 (II) (1). Therefore, a procedure capable of separating the *cis*- and *trans*-isomers in prostaglandin samples is needed.

A recent paper (2) described the high-pressure liquid

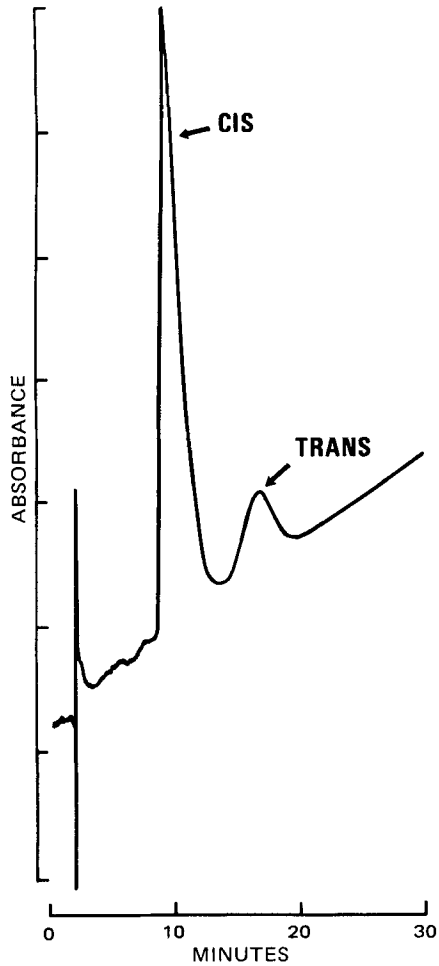

Figure 1—*Chromatogram of I and II methyl esters.*

chromatographic (HPLC) separation of a large number of prostaglandin *p*-nitrophenacyl esters using a microparticulate silica column. The partial separation of 5,6-*cis*- and *trans*-isomers of dinoprostone (III) and dinoprost (IV) was described, but no mention was made of the separation of prostaglandin A_2 or prostaglandin B_2 *cis*- and *trans*-isomers.

The separation of alkenes from saturated hydrocarbons (3, 4) and the resolution of *cis–trans*-isomers using silver-ion complexes are well-known procedures (5). The thermodynamics of alkene–silver-ion complexes in water show (6) that, although the enthalpy change favors formation, the entropy change is large and negative and causes the complex to be quite weak. Thus, temperatures near 25° are required for appreciable complexation. This low temperature requirement is easily satisfied by HPLC.

EXPERIMENTAL

Reagents and Samples—All solvents were distilled-in-glass quality[1], except water which was deionized. The anhydrous silver perchlorate[2] was used as received. The prostaglandin methyl esters[3] were used as received and stored as the methanol solutions.

Apparatus—The liquid chromatograph[4] was equipped with a 1-m column packed with 37-μm superficially porous beads with a bonded stationary phase of octadecylsilyl groups. Detection was by UV absorption at 254 nm. The mobile phase was 0.5 *M* $AgClO_4$ dissolved in methanol–water (20:80). The instrument was operated at 900 psi and room temperature. The sample concentrations were between 0.5 and 4 mg/ml, and 1–3-μl injections were used.

Because of the corrosive effect of the aqueous silver perchlorate on the copper drain tubing and on the brass pressure backup plate in the main pump, extensive plumbing changes were necessary. All copper drain tubing and brass fittings were replaced by perfluoropolyethylene tubing or stainless steel. The brass pressure backup plate was replaced by one made from ceramic-filled plastic.

The UV spectra were recorded on a recording spectrophotometer[5].

Mass spectra were taken on a high-resolution mass spectrometer[6] using an ionization potential of 70 v and direct probe sampling. Samples were prepared by evaporating an aliquot to dryness and reacting with reagent[7] to form the 15-trimethylsilyl derivative. Mass spectral samples from chromatographic cuts were obtained by running the aqueous chromatographic effluent into a flask containing about 10 ml of methylene chloride overlaid with about 3 ml of water. After each collection, the flask was immediately shaken to extract the prostaglandin into the organic phase. The phases were separated, and the methylene chloride fraction was washed with about 3 ml of water. The organic layer was taken to dryness and treated with derivatizing reagent as before.

Table I—Principal Ions in Mass Spectra of the 15-Trimethylsilyl Derivatives of I, II, and V Methyl Esters

Ion	*m/e* 15-Trimethylsilyl I and II Methyl Esters	*m/e* 15-Trimethylsilyl V Methyl Ester
$M^+\cdot$	420	420
$M^+\cdot - \cdot CH_3$	405	405
$M^+\cdot - \cdot C_2H_5$	391	391
$M^+\cdot - \cdot OCH_3$	389	389
$M^+\cdot - \cdot C_5H_{11}$	349	349
$M^+\cdot - (CH_3)_3SiOH$	330	330
$M^+\cdot - (\cdot C_5H_{11} + CO)$	321	321
$M^+\cdot - (\cdot C_5H_{11} + CH_3OH)$	317	317
—	199	247
—	190	

Table II—Comparison of Retention Times of 5,6-*cis*- and *trans*-Prostaglandin B_2 and Prostaglandin A_2 Methyl Esters

Compound	Retention Time, min
I Methyl ester	8.9
II Methyl ester	17.0
5,6-*cis*-V Methyl ester	16.4
5,6-*trans*-V Methyl ester	15.8

RESULTS AND DISCUSSION

Separation of I and II—Separation of I methyl ester from II methyl ester was achieved using a reversed-phase column[8] and a mobile phase of 0.5 *M* $AgClO_4$ in methanol–water (20:80). An example of the separation is shown in Fig. 1.

The retention times of the *cis*- and *trans*-isomers were 8.9 and 17.0 min, respectively. The *cis*-isomer eluted before the *trans*-isomer, because the complexation took place in the mobile phase and the stability constant of the silver–*cis*-isomer complex was greater (5). The relative sizes of the peaks were correct, since samples of I usually contained less than 15% of the *trans*-isomer.

An authentic sample of II methyl ester gave a single peak with a retention time of 17 min, which agreed very well with the peak assigned to the *trans*-isomer in Fig. 1.

[1] Burdick and Jackson Labs, Muskegon, Mich.
[2] G. F. Smith Chemical Co., Columbus, Ohio.
[3] Prepared by the Research Laboratory, The Upjohn Co., Kalamazoo, Mich.
[4] Model 820, DuPont Instrument Co., Wilmington, Del.
[5] Cary 15, Varian Inc., Palo Alto, Calif.
[6] Consolidated Electrodynamics Corp., Pasadena, Calif.
[7] Regisil, Regis Chemical Co., Morton Grove, Ill.
[8] ODS Permaphase, DuPont Instrument Co., Wilmington, Del.

Table III—Molar Absorptivities of Prostaglandin B_2 and Prostaglandin A_2 Methyl Esters

	Solvent 1[a]			Solvent 2[b]		
	λ_{max}	$\epsilon\lambda_{max}$	ϵ_{254}	λ_{max}	$\epsilon\lambda_{max}$	ϵ_{254}
Prostaglandin B_2	278	23,700	10,500	282	23,700	7560
Prostaglandin A_2	217	9,970	1,040	—[c]	—[c]	1520

[a] Solvent is methanol. [b] Solvent is 0.5 *M* $AgClO_4$ in methanol–water (20:80). [c] Data were not collectable due to solvent absorbance below 238 nm.

The possibility that the second, smaller peak of Fig. 1 was not the *trans*-isomer but an on-column degradation product of I methyl ester was checked by injecting a large amount, about 50 μg of the *cis–trans*-mixture, collecting the peak representing the *cis*-isomer, and reinjecting it. The reinjected material gave only one peak, and its retention time was the same as that for the *cis*-peak of the original mixture. Therefore, the chromatographic conditions had no apparent effect on the chemical stability of the samples and the peaks seen were not artifacts of the separation process.

A further check on the validity of the separation was carried out by injection of a biosynthetically produced I methyl ester. The material contains only the *cis*-isomer. Chromatographic analysis gave only one peak with a retention time of 9.2 min, in good agreement with the retention time of the *cis*-isomer in the mixture.

Mass Spectra of I and II Methyl Esters—The mass spectra of the pure biosynthetic I and pure II methyl esters were identical to each other and to the spectra of samples obtained by chromatographic cuts of elution curves. This finding is good evidence that the 8.9- and 17-min peaks differ only by a 5,6-*cis–trans*-relationship, since other prostaglandin A_2 methyl ester isomers or derivatives would be expected to give different mass spectra. The principal mass ions of the 15-trimethylsilyl derivative of I and II methyl esters are shown in Table I.

Chromatography of 5,6-*cis*- and *trans*-Prostaglandin B_2 Methyl Esters—Since I is often contaminated with prostaglandin B_2 (V), it was necessary to determine if the retention times of 5,6-*cis*- and *trans*-V methyl esters would interfere with the I or II methyl ester peak. Samples of the V methyl ester isomers[3] were chromatographed, and their retention times were close to each other and to the retention time of the II methyl ester (Table II).

Mass Spectra of V Methyl Esters—A chromatographic cut of 5,6-*cis*-V methyl ester was collected and submitted for mass spectral analysis as the trimethylsilyl derivative. Comparison with the mass spectra of the silylated unchromatographed 5,6-*cis*- and *trans*-V methyl esters showed no difference, indicating that the chromatographic process had no deleterious effect and that the samples of *cis*- and *trans*-V esters had the expected identical mass spectra. The principal mass ions of the 5,6-*cis–trans*-V methyl ester trimethylsilyl derivative are given in Table I. The 190 and 199 peaks in the prostaglandin A_2 series and the 247 peak in the prostaglandin B_2 series may be diagnostic of the differences among I, II, and V methyl esters.

UV Spectra of 5,6-*cis*- and *trans*-V, I, and II Methyl Esters—Additional evidence for the identity of the 5,6-*cis*- and *trans*-V, I, and II methyl esters was obtained from the UV spectra in methanol. The two samples of 5,6-*cis*- and *trans*-V methyl esters showed a peak at 278 nm and no evidence of a peak at 217 nm, indicating that neither sample contained any appreciable amount of I or II methyl ester. Conversely, the UV spectrum of II methyl ester, which had the same chromatographic retention time as the V isomers, indicated that the sample contained less than 5% of the V isomer. Approximately the same amount of V isomer was found in the biosynthetic I methyl ester.

The mass spectra and UV absorption data indicate that the assumed structures of the samples are correct. Therefore, the coincidence of the retention times of the *cis*- and *trans*-V methyl esters with the II methyl ester was real and not an artifact.

The UV spectra of V methyl ester in methanol–water (20:80), which was 0.5 *M* in silver perchlorate, showed a slight shift to a longer wavelength at the λ_{max} and no change in the molar absorptivity at the absorption maximum when compared to the spectrum in methanol. This finding indicates that the complexation of Ag^+ with I, II, and V methyl esters does not drastically change the chromophores. The molar absorptivities at the λ_{max} and at 254 nm are given in Table III.

Quantification of 5,6-*trans*-V Methyl Ester—An assay for the fraction of prostaglandin A_2 with the 5,6-*trans*-configuration is possible using the described procedure, but a reliable source of I methyl ester uncontaminated with the *trans*-isomer is needed to construct a calibration curve using a particular instrument and set of conditions. The 5,6-*cis*-isomer content can be found from HPLC data, and the sum of 5,6-*cis*- and 5,6-*trans*-isomers can be obtained from UV data. Therefore, the desired result can be calculated.

Possible Relationship of Structure to Lack of Separation of 5,6-*cis*- and *trans*-V Methyl Esters—A possible explanation as to why I and II methyl esters, but not the esters of 5,6-*cis*- and *trans*-V, can be separated in this system can be found in the differences in structure of prostaglandins B_2 and A_2. In prostaglandin A_2, the two side chains are *trans* to each other about the 8,12-bond. Thus, there is little or no interference of the lower side chain (C-13–C-20) with the approach of a silver ion to the 5,6-double bond. However, in prostaglandin B_2, the 8,12-double bond forces the side chains to be coplanar at the points of ring attachment. This orientation could result in a serious interference by the C-13–C-20 side chain with the approach of a silver ion to the 5,6-unsaturation. If no complexation occurs, there can be no separation of 5,6-*cis*- and *trans*-V esters, as is observed.

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ACKNOWLEDGMENTS AND ADDRESSES

Received March 25, 1976, from the *Research Laboratories, The Upjohn Company, Kalamazoo, MI 49001.*

Accepted for publication July 6, 1976.