Dehydration of prostaglandins: study by spectroscopic method

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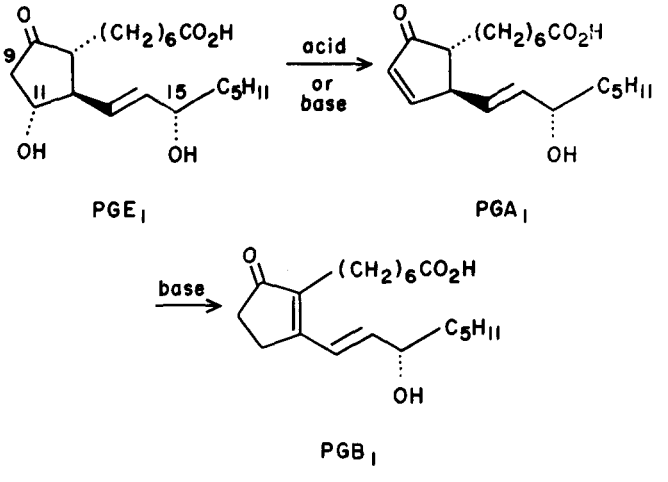
ABSTRACT The ultraviolet (UV) and optical rotatory dispersion (ORD) spectra of prostaglandins E_1, A_1, B_1, and their naturally derived 15-epimers are presented. The dehydration sequence E → A → B under acidic and basic conditions has been studied by UV and ORD. Conditions for quantitative conversion of PGE_1 to PGA_1 are described.

The combination of ORD and UV affords a nondestructive assay which can determine the relative amounts of E-, A-, and B-type prostaglandins with as little as 1–2 μg of material. The total quantity of prostaglandins can be estimated (±15%) in this way or, more accurately, by treatment with alkali of an aliquot (200 ng is sufficient) and determination of the change in absorbance at 278 nm.

SUPPLEMENTARY KEY WORDS prostaglandins · spectroscopic assay · UV spectra · optical rotatory dispersion · β-hydroxyketones

When they are available only in minute amounts, the prostaglandins are generally assayed by their action on smooth muscle tissues or by vasodepressor activity. Although the various types of prostaglandins in a mixture can be differentially assayed by utilization of their differing activities on various tissues, the determinations are inaccurate because of poor reproducibility due to general experimental difficulties and the low slope of dose–response curves.

In the general method for quantification of 9-keto prostaglandins the molecule is dehydrated and then induced to rearrange in the presence of alkali to give the conjugated dienone PGB_1 (ϵ_{278} = 28,650) (2). I have studied this sequence under both acidic and basic conditions by UV absorption and optical rotatory dispersion (ORD) with the aim of developing accurate and sensitive spectroscopic assays for the prostaglandins.



METHODS

The solvents and reagents used were the purest available commercial products (Mallinckrodt spectrograde or nanograde).

The prostaglandins used were judged pure by TLC (1) with the exception of $PG(E\alpha\beta)_1$, which contained 20–25% of $PG(A\Delta\beta)_1$ due to dehydration on standing. The amount available was too small to purify.

UV spectra were recorded on a Perkin–Elmer model 202 or Cary model 14 spectrophotometer. In general, cylindrical cells of 1 cm path length and 3 ml volume were used. The spectra were recorded at the operating

Abbreviations: THF, tetrahydrofuran; ORD, optical rotatory dispersion; TLC, thin-layer chromatography. An abbreviated form of the systematic nomenclature for the prostaglandins is used. In this system [more fully explained elsewhere (1)] the configuration of the hydroxyl groups at the 11- and 15-positions is indicated by α or β. Thus $PG(E\alpha\beta)_1$ is 15-epi-PGE_1, the 15-epimer of naturally occurring PGE_1, for which the corresponding notation is $PG(E\alpha\alpha)_1$. $PG(A\Delta\beta)_1$ is the 15-epimer of PGA_1; Δ indicates unsaturation at C-11.

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temperature (about 30°C). The ORD spectra were recorded on a Cary 60 recording spectropolarimeter with cylindrical cells having a volume of 3.0 ml and a path length of 1.00 cm. Microcells having a 1.00 cm (height) × 0.10 cm × 1.00 cm (path length) cavity gave the same results.

Prostaglandin Assay by UV Determination of PGB_1 Formed

An aliquot (5–60 μl of a methanol, ethanol, or dioxane solution) containing 10–50 μg of prostaglandins was diluted to 3.00 ml with methanol. The UV spectrum was taken in order to determine the initial PGB concentration. Concentrated aqueous KOH (15–50 μl of 8.1 N solution) was added (final solution 0.04–0.12 N). The UV spectrum was scanned in the 220 and 280 nm regions immediately (0.5–1.0 min) after the addition of base and intermittently over the next 30–90 min. Absorbances at 218 and 278 nm were plotted against time. A ΔA_{278} value of 1.00 indicates 40 μg of PGE_1 (or its equivalent of PGE isomers plus PGA isomers) in the aliquot [see similar value, 39.4 μg, for 0.5 N methanolic KOH (3)]. The assay can be carried out with as little as 50 μl (total volume) in the microcell; thus 67 ng of PGE_1 can be estimated with a precision of ±5% (ΔA_{278} = 0.100 ± 0.005).

The *rate* of PGB_1 formation from the diastereomeric PGE compounds was determined as above but the cell and its contents were allowed to come to thermal equilibrium before the KOH was added. After addition of base the absorbance at 278 nm was measured every 30 sec. The temperature during the runs was about 30°C. The probable relative error in the times listed in Table 1 is ±12 sec; the absolute error may be greater due to the imprecision in determining the temperature at which the measurements were taken.

ORD Study of the Dehydration of $PG(E\alpha\alpha)_1$ and $PG(E\alpha\beta)_1$ Under Basic Conditions

A dioxane solution (15 μl) of the prostaglandin[1] (0.02 μmole/μl) was added to a 3.00 ml cell, which was then filled to volume with methanol to give a solution containing 0.10 μmole of PGE/ml of solution. The ORD was recorded in the usual way. Then 15 μl of 8 N aqueous KOH was added. After mixing, the cell was placed in the beam (at 25 ± 1°C), and the ORD was scanned periodically (typical curves are shown in portions *A* and *C* of Fig. 3, below).

[1] Authentic PGE_1 was the gift of Professor Bengt Samuelsson of the Karolinska Institutet (Stockholm) and 15-epi-PGE_1 from natural sources was the gift of Dr. W. P. Schneider of the Upjohn Co. PGA_1, PGB_1, and $PGF_1\beta$ were prepared from authentic PGE_1 by known methods (2) or as described in this communication. The racemic compounds were prepared from totally synthetic *dl*-PGE_1 (4, 5).

TABLE 1 PGB_1 FORMATION IN 0.11 N KOH/CH_3OH*

% Conversion	$PG(E\alpha\alpha)_1$	$PG(E\alpha\beta)_1$	$PG(E\beta\alpha)_1$
		min	
25	2.85	2.85	3.80
50	5.15	5.20	6.40
75	8.05	8.15	9.60
90	11.25	11.75	13.35

* 24.5 ± 1 μg of PGE per 3.0 ml cell.

The molar optical rotations, [Φ], observed for the impure $PG(E\alpha\beta)_1$ sample were: initially, 350 nm (−2300°), 313–317 nm (−6900°, trough), 298.5 nm (0°), 270 nm (+13,000°, peak), 245 nm (+11,300°, shoulder), 235 nm (+8,000°, sh), 230 nm (+6,000°), and 220 nm (+700°); at 16–20 min, 320 nm (0°), 287 nm (+9,600°, sh), 251 nm (+20,400°, peak); at 90–100 min, 380 nm (−150°), 350 nm (−700°), ~292 nm (−3,800°, trough). Because of the impurity of the sample, these rotations should not be considered as definitive values.

Acid-Catalyzed Dehydration of PGE_1

A stock dioxane solution of PGE_1 was used (2.83 μg/μl). A 12 μl aliquot was added to 1 ml of one of the following acidic mixtures and the mixture was treated as described.

(*a*) Water–acetic acid–85% aq H_3PO_4 10:3:2; kept at room temperature for 9 hr.

(*b*) As in (*a*); then heated at 70°C for 1 hr.

(*c*) THF–1 N aq HCl 1:1; kept at room temperature for 60 hr.

(*d*) Buffer, made by addition of 5 ml of 85% aq H_3PO_4 to 100 ml of acetone–water–acetic acid 10:4:1; kept at room temperature for 60 hr.

The reaction mixtures were all then handled in the same way: 10 ml of water was added, the mixture was extracted with two 8 ml portions of ether, and the ether extracts were washed with two 10 ml portions of saturated aq sodium chloride, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Dilution of the residual oil to 10.0 ml with methanol gave the solution used for ORD (Table 3, below) and UV studies.

An aliquot of each reaction mixture (methanol solution from above) was concentrated for TLC [neutral silica, system *F*-I (1)]. PGE_1 (R_f 0.21) was entirely consumed after procedures *b*–*d*, but 20% PGE_1 remained after procedure *a*. The PGA_1 spot (R_f 0.42) predominated in all reaction mixtures except *b*, which showed PGA_1 and a strong companion spot with slightly higher R_f (0.44). Samples *c*, *b*, and especially *d* showed faster-running contaminants in the R_f 0.55–0.7 region.

Prostaglandin Assay by ORD Determination of PGA Formation

The prostaglandin sample in a limited volume of

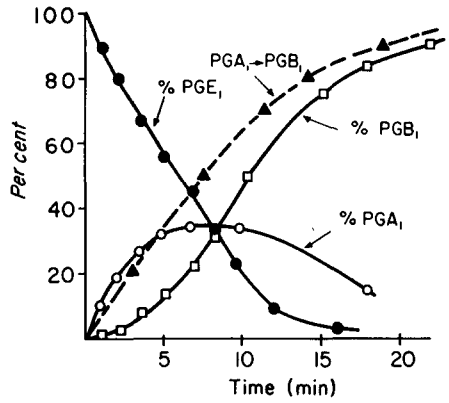

FIG. 1. The reaction profile for the dehydration of PGE_1 in 0.041 N methanolic KOH (solid lines), and the rearrangement of PGA_1 to PGB_1 in the same medium (▲--▲).

dioxane was added to the cell containing mixture *a* (above). The ORD was scanned immediately after mixing and then after 15 hr at 25°C. Authentic PGE_1 gave an apparent $[\Phi]_{255}$ of +31,000° under these conditions. Alternatively, the cell and its contents can be kept in a waterbath at 70°C for 40 min. The cell was removed at 15, 20, 25, 30, and 40 min, and cooled for ORD determination. Both PGE_1 and $PG(E\alpha\beta)_1$ give an apparent $[\Phi]_{255}$ = +30,000° between 20 and 30 min (see Fig. 3, portions *B* and *D*).

The relationship between observed rotation and quantity of 9-keto prostaglandins for a cell of 1 ml volume and 1.0 cm path length is: 10 nmoles = +0.0031° observed.

RESULTS AND DISCUSSION

Pure PGE_1 (4, 5) shows only end absorption (ϵ_{210} = 830, ϵ_{218} = 245), but PGA_1 (ϵ_{218} = 10,900) (2) could, in principle, be estimated from the absorption at its UV maximum. However, most samples, particularly those of biological origin, have interfering absorptions at this low wavelength.[2]

The original procedure (6) for conversion of either PGE_1 or PGA_1 to PGB_1 (λ_{max} = 278 nm) employed ethanol–1 N NaOH 1:1 and gave $\Delta\epsilon_{278}$ = 23,000. Under these conditions any changes at 218 nm cannot be determined, because of the high absorption of the blank. We have found that convenient rates of conversion are obtained with 0.04–0.12 N methanolic KOH. The assay gives $\Delta\epsilon_{278}$ = 27,200 ± 500 for both PGE_1 and PGA_1; thus the conversion is complete. At prostaglandin concentrations of 5–20 μg/ml the assay requires 0.5–1.5 hr, depending on the concentration of KOH. The absorbances at 218 and 278 nm give the composition of the reaction mixture at any time. The "reaction profile" of PGE_1 in 0.041 N methanolic KOH is shown in Fig. 1. The characteristics are $t_{50\%}$ (278 nm) = 10.3 min and %-PGA_{max} = 35%. Epimeric prostaglandins show different characteristics. The dashed line (▲- - -▲) shows the conversion of PGA_1 [$t_{50\%}$ (278 nm) = 7.5 min] under the same conditions.

[2] Solutions of PGE_1 are not stable: the formation of PGA_1 can be followed by UV or ORD spectra. For example, we found that methanolic PGE_1 (40 μg/ml) was 5–20% converted to PGA_1 after 1 month at 5–10°C.

TABLE 2 ORD OF PROSTAGLANDINS

	Medium	Concentration	Ref.	Wavelength and Molar Optical Rotation
		g/100 ml		*nm* (°)
PGE_1	Methanol	0.14	7	315 (−7500°, trough), 276 (+7300°, peak)
	Methanol	0.00354	*	311–318 (−7000°, trough), 296.5 (0°), 236 (+6000°, trough)
	Dioxane	0.0868	2	320 (−7200°, trough), 300–305 (sh), 275 (+6600°, peak), ~237 (trough)
	Water	0.0076	*	307 (−8600°, trough), 288 (0°), 265.5 (+8600°, peak), ~228 (5100°, trough)
	WAP†	0.0067	*	307 (−8000°, trough), 290 (0°), 267.5 (+10,200°, peak),† 245 (8200°)‡
PGA_1	Water	0.0040	2	330–345 (+500°, broad trough), 300 (+13,200°, sh), 256 (+35,000°, peak ~239 (0°), 220 (−43,000°), trough)
	Methanol§	~0.001	*	290–295 (sh.), 250 (~34,000°, peak), ~232 (0°), 218 (−43,500°, trough)
	WAP†¶	0.0067	*	~340 (trough), ~290 (sh), 255 (~33,000°, peak)
PGB_1	Methanol‖	0.00354	*	340 (+400°), 310 (+1100°), 292 (+3900°, peak), ~250 (~−2800° trough)
$PGF_1\beta$	Methanol	~0.02	*	Very low rotation with negative trend: 235 (~ − 500°)

sh, shoulder.

* Present work.

† Water–acetic acid–85% aq H_3PO_4 10:3:2. The rotation value given reflects partial conversion to PGA_1 during the required time for recording.

‡ The absorbance below 245 nm was too high for reliable rotation measurements.

§ ORD of crude product from acidic dehydration of PGE_1. Quantified by UV assay (PGB_1 formation).

¶ PGA_1 generation in situ from PGE_1. Rotations based on amount of PGE_1 employed.

‖ The PGE_1 solution was treated with aqueous potassium hydroxide to give a 0.04 N alkali solution. Only PGB_1 remains after 90 min. The extremely low rotation and high absorbance of the sample made the determination of optical rotation between 290 and 260 nm impossible.

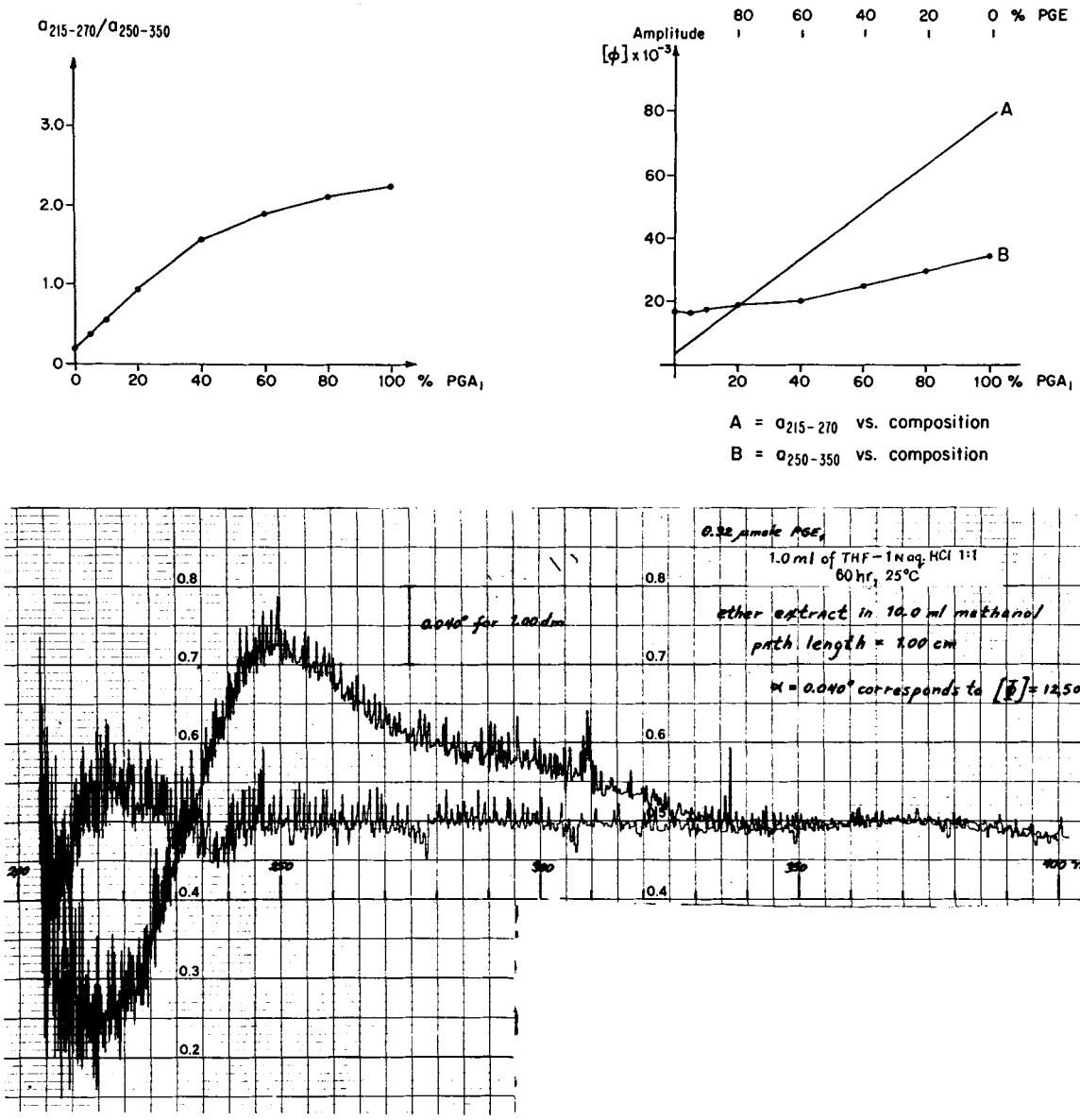

FIG. 2. A sample ORD spectrum for PGA_1 (8.9 μg PGA_1/ml, 1.00 cm path); graphed relationships between the observed ORD amplitudes and the composition of mixtures of PGE_1 and PGA_1.

Synthetic *rac*-PG(E$\alpha\beta$)$_1$ and *rac*-PG(E$\beta\alpha$)$_1$ (15-epi- and 11-epi-PGE_1 respectively) were also investigated (4, 5). The rate of conversion to PGB_1 is virtually unaffected by the changed C-15 configuration, but the 11β-compounds appear to dehydrate more slowly (Table 1).

One point concerning UV assay should be emphasized: quantification of PGE_1 and PGA_1 by UV determination of PGB_1 formed is more accurate if the difference between final absorbance and absorbance at zero time after base addition, ΔA_{278}, rather than final A_{278}, is used.

With the general availability of sensitive recording spectropolarimeters, ORD should offer another nonbiological assay. The ORD spectra of PGE_1, PGA_1, PGB_1, and $PGF_1\beta$ are summarized in Table 2. The rotations are not unusually high, but they are large enough to allow quantification of solutions containing as little as 4 μg/ml of prostaglandins. The actual tracing for PGA_1 prepared from PGE_1 by acid dehydration is shown in Fig. 2. UV assay by PGB_1 formation indicated that the solution used contained 8.9 μg PGA_1/ml. Cells with a 0.25 ml volume and a path length of 1.0 cm could be used without modification of the spectropolarimeter. The use of a beam condenser and other modifications probably will extend the method so that 200 ng of prostaglandins can be detected.

Mixtures of PGE_1 and PGA_1 show a peak at 250–265 nm and troughs at 215–230 and 307–340 nm. The two

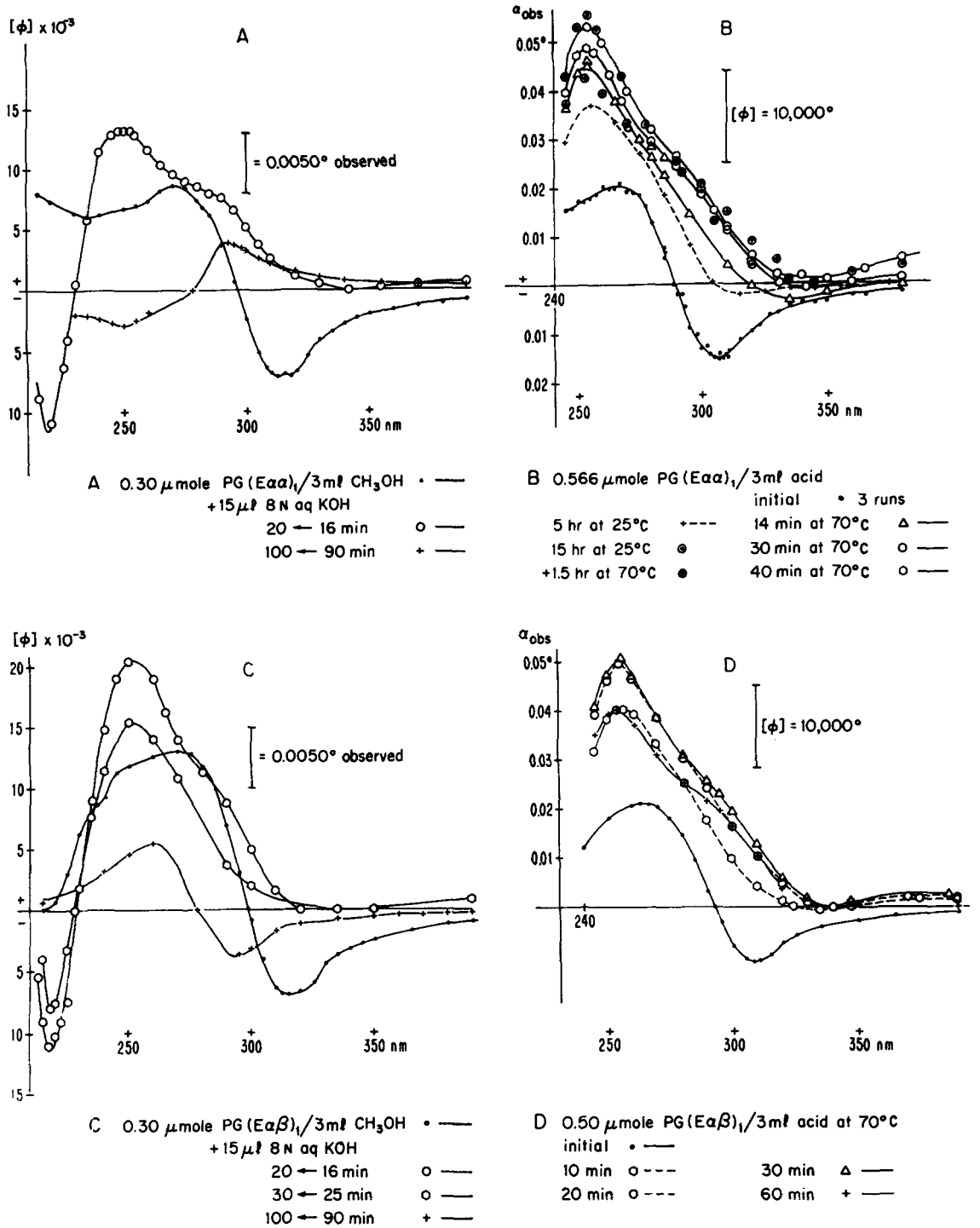

FIG. 3. ORD curves of PG(Eαα)$_1$ and PG(Eαβ)$_1$, before, during, and after dehydration under basic (*A* and *C*) and acid (*B* and *D*) conditions.

measurable amplitudes ($a_{215-270}$ and $a_{250-350}$) can be used separately or as a ratio to determine the composition of the mixture. These correlations are shown in graphs on Fig. 2.

The dehydration rearrangement of PG(Eαα)$_1$ and PG(Eαβ)$_1$ under basic conditions can be brought about in the ORD cell. The spectra, recorded at various times after the addition of base, are shown in *A* and *C* of Fig. 3; they confirm the reaction course determined by UV. The strong rotation of the PGA chromophore (at 250 nm) is the predominant contributor to the ORD curve during the course of the reaction even though PGA

TABLE 3 ACID-CATALYZED DEHYDRATION OF PGE_1

Run*	PGB formation†	$[\Phi]_{max}$‡	$[\Phi]_{min}$‡
	%		
A§	92	+31,000°	−29,000°
B	51	+29,000°	−26,000°
C	83	+28,000°	−36,000°
D	71	+28,000°	−31,000°

* The four sets of reaction conditions are given as *a–d* in Methods.

† % Recovery (relative to initial amount of PGE_1) of PGB-forming material, from another aliquot of solution used for ORD assay.

‡ Rotation based on amount of PGE_1 used. The ORD had the general shape of that of PGA in each case. The product from *c* appeared to be the most nearly pure. Rotations (for C) based on the UV assay are: $[\Phi]_{250} = +34{,}000°$ and $[\Phi]_{218} = -43{,}500°$, in excellent agreement with the literature values for pure authentic PGA_1.

§ The ORD suggested the presence of 20–25% PGE_1, which was confirmed by TLC.

makes up only 35% of the mixture at this stage. The $PG(E\alpha\beta)_1$ sample employed contained some $PG(A\Delta\beta)_1$ (~10%), and thus the exact rotation for this isomer cannot be given. The curves suggest that the trough at 315 nm, $[\Phi] = -7000°$, is identical with that of PGE_1 [the naturally derived 15-epimer of $PG(E\alpha\beta)_1$]. However, the peaks of $PG(E\alpha\beta)_1$ and $PG(A\Delta\beta)_1$ appear to be more positive than those of PGE_1 and PGA_1, which suggests a positive contribution for the 15β-hydroxyl on the natural prostane skeleton. After 90 min, only PGB remains. The ORD curves of PGB obtained from the two 15-epimers show, within experimental error, the expected mirror-image relationship. The unnatural 15(*R*)-enantiomer has a positive rotation below 270 nm.

Finally, the acid-catalyzed conversion of E-type prostaglandins to A-type prostaglandins was studied in the hope of using the higher rotation of the A chromophore in a more sensitive assay. Authentic PGE_1 was treated by procedures *a–d* (see Methods). The isolated product was assayed by PGB_1 formation and examined by ORD. The results are summarized in Table 3 Clearly, PGA_1 was formed under conditions *a* and *c;* under conditions *b* and *d*, material that resembles PGA_1 by ORD but does not give PGB_1 on base treatment was formed in addition to true PGA_1.[3] Treatment under conditions *a* for 15–20 hr has since been found to give nearly quantitative conversion to PGA_1 and is the basis of a sensitive ORD assay for prostaglandins. The ORD curves $PG(E\alpha\alpha)_1$ and $PG(E\alpha\beta)_1$ obtained by this new assay are shown in B and D of Fig. 3. The rotation at 254–256 nm reaches $[\Phi] = +31{,}000°$ after 15 hr at room temperature or after 20–30 min of heating at 70°C. Assuming that the dehydration rates of 11-epimeric and polyunsaturated prostaglandins do not differ greatly, this assay should be applicable to all 9-ketoprostaglandins having the natural configuration at C-8 and C-12; the enantiomeric forms should give $[\Phi]_{255} = -31{,}000°$.

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[3] TLC system *F*-I (1) showed PGA_1 in all four samples. Sample A (see Table 3) showed PGE_1 as well. Sample B showed an equally dense spot running just ahead of PGA_1 as well as at least one more mobile spot. Sample D also showed more mobile spots.