15-Hydroxy-9-oxoprosta-11,13-dienoic acid as the product of a prostaglandin isomerase

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Abstract The initial product of the interaction between prostaglandin A_1 and the prostaglandin isomerase of cat blood plasma has been isolated. By ultraviolet spectroscopy and mass spectrometry and from stability and chromatographic studies, the structure of the compound has been established as 15-hydroxy-9-oxoprosta-11,13-dienoic acid, an allylic isomer of prostaglandin A_1. The compound is unstable under mild alkaline conditions, isomerizing to prostaglandin B_1. The biological significance of the enzymatic isomerization of prostaglandin A_1 is discussed.

Supplementary key words prostaglandins · enzymatic isomerization · keto–enol tautomerism · conjugated diene

PROSTAGLANDINS OF THE A series are naturally occurring fatty acids with potent actions on renal sodium excretion, gastric acid secretion, and peripheral vascular resistance (1, 2). Recently it has been shown that the incubation of prostaglandin A_1[1] with blood plasma of the cat results in the formation of the biologically inactive, isomeric prostaglandin B_1 (3, 4). The conversion was found to be enzymatic in nature and the enzyme system was given the tentative name of prostaglandin isomerase. Further studies have shown that the true product of this enzyme reaction is an isomer intermediate in structure between prostaglandins A_1 and B_1, and that prostaglandin B_1 arises due to the instability of the intermediate isomer under mild alkaline conditions. The detection, isolation, and characterization of this compound are the subject of the present paper.

EXPERIMENTAL PROCEDURES

Preparation of prostaglandins A and B

Prostaglandins A_1, ω-homo, and α-nor A_1 were prepared by dehydration of the corresponding prostaglandin E with 0.5 N HCl in 50% aqueous tetrahydrofuran (5). Prostaglandin A_1 was purified by reversed-phase partition chromatography. The support material was hydrophobic Hyflo Super-Cel, and the solvent system (F55) consisted of a moving phase of methanol–water–acetic acid 165:135:2 and a stationary phase of chloroform–*n*-heptane 45:5 (6). ω-Homo prostaglandin A_1 and α-nor prostaglandin A_1 were purified by silica gel thin-layer chromatography using the solvent system toluene–dioxane–acetic acid 20:20:1.

Prostaglandins B_1 and B_2 were prepared from the corresponding prostaglandins A by treatment with 0.1 N KOH in methanol at room temperature for 90 min (5).

Isolation of intermediate isomer

0.06 mmole of prostaglandin A_1 in 4 ml of methanol was added to 6 mU of prostaglandin isomerase[2] (purified 20-fold from cat plasma) in 56 ml of 0.1 M Tris–HCl buffer (pH 7.0). The temperature of the reaction mixture was maintained at 25°C. At 20-min intervals, 0.05-ml aliquots were removed and diluted with 2 ml of methanol in a glass cuvette, and an ultraviolet extinction spectrum between 200 and 325 nm was recorded. After 90 min the reaction mixture was cooled to 0°C and applied to a column (40 × 600 mm) of G-25 Sephadex gel maintained

[1] Full chemical names: prostaglandin A_1, 15S-hydroxy-9-oxoprosta-10,13-*trans*-dienoic acid; prostaglandin A_2, 15S-hydroxy-9-oxoprosta-5-*cis*,10,13-*trans*-trienoic acid; prostaglandin B_1, 15S-hydroxy-9-oxoprosta-8(12),13-*trans*-dienoic acid; prostaglandin E_1, 11α,15S-dihydroxy-9-oxoprost-13-*trans*-enoic acid.

[2] A Unit of prostaglandin isomerase has been defined as the amount of enzyme that will catalyze the formation of 1 μmole of prostaglandin B_1/min under the following conditions: substrate, 0.2 μmole prostaglandin A_1; end volume, 3.0 ml; buffer, 0.1 M Tris–HCl (pH 8.5); temp, 25°C. (Jones, R. L., S. Cammock, and E. W. Horton. Unpublished results.)

between 0 and 4°C. Elution was performed with Tris buffer (pH 7.0) at 8 ml/min, and 24-ml fractions were collected. Ultraviolet spectra of fractions with elution volumes of 500–900 ml were obtained (previous experiments with this size of column had shown that low molecular weight substances were eluted in this region). Those fractions exhibiting a significant extinction at 234 nm were combined (650–810 ml), acidified to pH 5.5 with HCl, and partitioned twice with an equal volume of diethyl ether. The combined ether phases were washed with water and evaporated to dryness. The residue was purified by reversed-phase partition chromatography, using 6.75 g of Hyflo Super-Cel and solvent system F55. The fractions containing the intermediate isomer were combined and evaporated to dryness. The residue was suspended in *n*-hexane and stored at −20°C.

Repurification of intermediate

The initial sample of the intermediate isomer containing some prostaglandin B_1 formed on storage was applied as a band to a 5 × 20 cm thin-layer chromatography plate coated with a 0.5-mm layer of silica gel G. Authentic prostaglandins A_1 and B_1 and a small portion of the mixture were applied as spots to a second plate. Both plates were developed simultaneously in the same tank using the solvent system toluene–dioxane–acetic acid 50:30:1. The marker plate spots were visualized by spraying the plate with 10% phosphomolybdic acid in ethanol followed by heating at 110°C for 10 min. The silica gel from the 0.38–0.44 R_F zone of the preparative plate (containing the intermediate isomer only) was removed and eluted with methanol. The methanol was diluted with 50 vol of water and extracted with diethyl ether. The ether was evaporated and the residue was stored dry, under nitrogen, at −20°C.

Identification of intermediate

Ultraviolet spectroscopy. Ultraviolet spectra were recorded on a Pye Unicam SP 800 spectrophotometer. Repeated scanning was performed with an SP 825 program controller. The cuvettes, of 10-mm path length, were enclosed in a constant-temperature housing maintained at 25 ± 0.3°C. The molar extinction coefficient (ϵ_{max}) of the intermediate was estimated as follows: 1.9 ml of a methanolic solution of the intermediate (calculated to contain approximately 15 μg/ml) was placed in a cuvette, and the extinction at 234 nm was measured. 0.1 ml of 0.02 N KOH in methanol was then added, and the final extinction at 278 nm due to prostaglandin B_1 (λ_{max}^{MeOH} = 278 nm) was recorded. The ratio of the recorded extinctions (corrected for the volume change) multiplied by the molar extinction coefficient of prostaglandin B_1 gives the molar extinction coefficient of the intermediate.

Infrared spectroscopy. Prostaglandins were applied to an NaCl disc in 0.1 ml of freshly redistilled chloroform. Spectra were recorded with a Perkin-Elmer 237 infrared spectrophotometer.

Mass spectrometry. The methoxymes of prostaglandins A_1 and B_1 were prepared by treatment with 1% methoxylamine HCl in dry pyridine at 60°C for 2 hr (7). The intermediate was reacted with 1% methoxylamine HCl in 1 M sodium acetate buffer (pH 4.0) containing 20% ethanol at 60°C for 1 hr to form the methoxime. Methyl esters of the prostaglandins were prepared by treatment with ethereal diazomethane solution at room temperature for 15 min.

Mass spectra of both the methyl esters and the methyl ester-methoximes of the prostaglandins were obtained using the direct insertion probe of an LKB 9000 mass spectrometer. The probe was heated at a rate of 5°C/min and mass spectra were recorded at 27.5 ev.

RESULTS

Detection of intermediate

After the addition of prostaglandin A_1 ($\lambda_{max}^{H_2O}$ = 222.5 nm) to a dilution (1:100) of cat blood plasma at pH 8.5, the slow appearance of a chromophore with an ultraviolet extinction maximum at 283 nm can be detected by repeated scanning between 250 and 350 nm. The new chromophore is due to the production of prostaglandin B_1 ($\lambda_{max}^{H_2O}$ = 283 nm) (Fig. 1) by prostaglandin isomerase (3, 4). When a partially purified preparation of this enzyme became available, it was possible to record spectral changes accurately in the region below 250 nm after addition of prostaglandin A_1. The rapid appearance

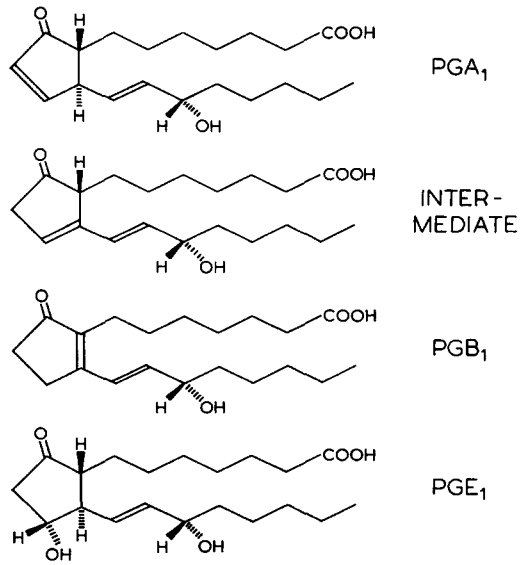

FIG 1. Formulas of prostaglandin A_1, the postulated intermediate, prostaglandin B_1, and prostaglandin E_1.

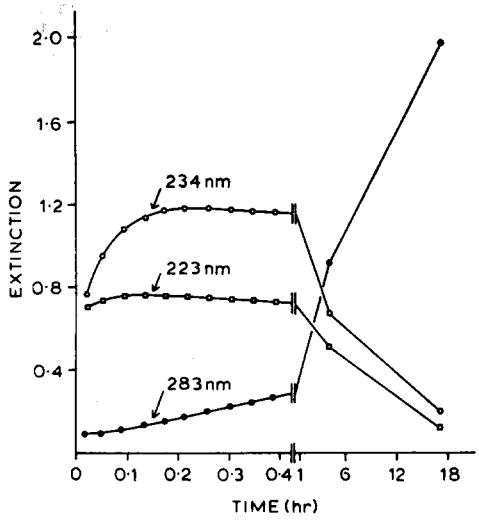

FIG. 2. Ultraviolet spectral changes after the addition (at time 0) of 0.02 μmole of prostaglandin A_1 to 0.3 mU of prostaglandin isomerase contained in 3.0 ml of 0.1 M Tris–HCl buffer (pH 8.5) at 25°C.

of an absorption with a maximum at 234 nm, preceding any significant increase at 283 nm, was thus observed (Fig. 2). This 234-nm absorption reached a maximum after 10–15 min and then slowly declined while absorption at 283 nm increased. After overnight incubation (17 hr), only the 283-nm chromophore remained. No spectral changes between 210 and 350 nm were observed when prostaglandin A_1 was incubated in the absence of enzyme at pH 8.5 for 17 hr. The incubation of prostaglandin A_1 at pH 12, again in the absence of the isomerase, results in the formation of prostaglandin B_1 without the prior appearance of a 234-nm chromophore.

These results suggested that prostaglandin A_1 is converted by prostaglandin isomerase to a compound with an extinction maximum at 234 nm (termed the intermediate) which in turn gives rise to prostaglandin B_1.

Isolation of intermediate

The intermediate was prepared by incubating 20 mg of prostaglandin A_1 with prostaglandin isomerase at pH 7.0 and 25°C. At this pH the amount of prostaglandin B_1 formed is very small. When the concentration of the intermediate (estimated from the 234-nm extinction) had reached a maximum, the reaction was stopped by cooling to 0°C. The prostaglandins were separated from the proteins of the prostaglandin isomerase extract by rapid gel filtration, and they were extracted into ether. Reversed-phase partition chromatography was used to separate prostaglandins A_1 and B_1 from the intermediate (Fig. 3). Prostaglandin A_1 was eluted first, closely followed by prostaglandin B_1. The moving phase was then made less polar by the addition

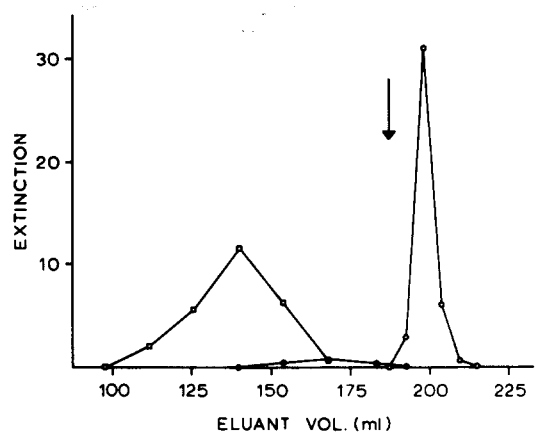

FIG. 3. Reversed-phase partition chromatography of residue from the ether extraction (see text for details; support, 6.75 g of Hyflo Super-Cel; solvent system, F55; flow rate, 7 ml/hr). Ordinate, ultraviolet extinction of individual fractions at 217 nm (□, corresponding to prostaglandin A_1), 234 nm (○, intermediate compound), and 278 nm (●, prostaglandin B_1). Abscissa, eluant volume in ml. At the arrow the moving phase was made less polar by the addition of 10% methanol.

of 10% methanol, and immediate elution of the intermediate was obtained. The yield of the intermediate was 4.2 mg.

Properties of the intermediate in comparison with those of prostaglandins A_1 and B_1

Ultraviolet spectroscopy. Ultraviolet spectral data for prostaglandins A_1 and B_1 and the intermediate are presented in Fig. 4 and Table 1. The intermediate in methanol exhibits an extinction maximum at 234 nm, with shoulders occurring at 228 and 243 nm. The latter are more pronounced in hexane. The wavelength of maximum absorption of the intermediate remains constant on changing the solvent from *n*-hexane to water. In contrast, prostaglandins A_1 and B_1 exhibit pronounced red shifts of 9.5 and 15 nm, respectively, on changing from *n*-hexane to water.

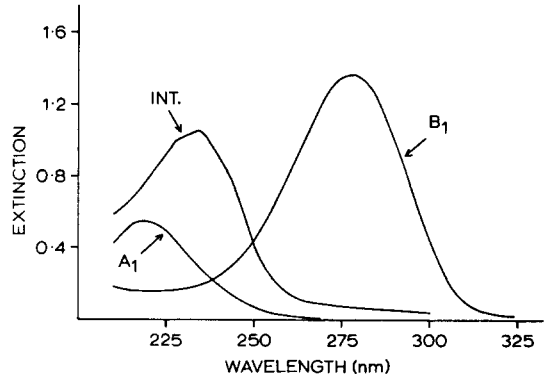

FIG. 4. Ultraviolet spectra of prostaglandins A_1 and B_1 and the intermediate compound in methanol. Concentrations are 0.05 mM and the path length is 10 mm.

TABLE 1. Ultraviolet spectral data for prostaglandins A_1 and B_1 and the intermediate isomer

	Wavelength of Maximum Absorption			
	n-Hexane	Methanol	Water (pH 7.0)	As Methyl Ester-methoxime in *n*-Hexane
		nm		
PGA_1	213	218.5 (10,900)[a]	222.5	244
PGB_1	268	278 (28,650)[a]	283	277
Intermediate isomer	235	234 (21,000)	234	237

[a] These values (in parentheses) are molar extinction coefficients as quoted by Andersen (5).

TABLE 2. Mass spectral data for prostaglandins A_1, A_2, ω-homo A_1, and α-nor A_1 as the methyl ester derivatives

	% Abundance at *m/e* Value					
	107	119	133	147	190	204
PGA_1	28	45	27	9	65	34
PGA_2	35	69	52	18	100	6
ω-homo PGA_1	15	19	16	7	7	31
α-nor PGA_1	38	48	37	18	70	28

The conversion of the 9-oxo function of prostaglandins A_1 and B_1 to a methoxime results in a shift of the extinction maximum to a longer wavelength and a broadening of the extinction spectrum. The spectrum of the methoxime derivative of the intermediate shows a maximum at 237 nm, retention of the vibrational fine structure, and no broadening of the peak profile.

A value of 21,000 was obtained for the molar extinction coefficient of the intermediate in methanol.

Mass spectrometry. Mass spectra of the methyl esters of prostaglandins A_1 and B_1 and the intermediate are shown in Fig. 5. Mass spectra of the methyl esters of prostaglandins A_2, ω-homo A_1, and α-nor A_1 were also obtained, and the relevant data are presented in Table 2. The methyl ester-methoxime derivatives of prostaglandins A_1 and B_1 and the intermediate exhibited molecular ions at *m/e* 379. The prostaglandin A_1 spectrum showed a base peak at *m/e* 148 and prominent ions at *m/e* 99 (30%), 71 (25%), and 43 (22%). The intermediate spectrum showed prominent ions at *m/e* 280 (100%), 99 (95%), 71 (52%), and 43 (37%); the peak at *m/e* 148 was only 8% of the base peak.

Stability. In weakly alkaline solution the intermediate is quickly converted to prostaglandin B_1, as evidenced by the disappearance of the 234-nm chromophore and the concomitant appearance of a 278-nm chromophore. Thus, on incubation with 0.01 N KOH in methanol at 25°C, 85% conversion to prostaglandin B_1 occurs in 1 min. This conversion can be detected in aqueous solution, even at pH 7; a comparable rate of production of prostaglandin B_1 from prostaglandin A_1 requires a pH between 11.5 and 12.

Infrared spectroscopy. Prostaglandin A_1 showed infrared extinction maxima at 1710 (α,β-unsaturated carbonyl in 5-membered ring), 1590 (double bond conjugated with carbonyl), and 970 cm^{-1} (*trans* double bond), in close agreement with the previously published values (8). The spectrum of the intermediate revealed a somewhat ill-defined, broad extinction band between 1680 and 1760 cm^{-1} and two other bands at 800 and 1260 cm^{-1}. On dissolution of the NaCl disc in water, it was found by ultraviolet spectroscopy that at least 40% of the intermediate had undergone conversion to prostaglandin B_1 during its stay on the disc.

Chromatographic properties. The intermediate is only slightly less polar than prostaglandins A_1 and B_1 on both reversed-phase partition chromatography (Fig. 3) and silica gel thin-layer chromatography. The intermediate appeared as a single spot with an R_F of 0.41; prostaglandins A_1 and B_1 had R_F values of 0.38 and 0.37, respectively. Thin-layer chromatography provided an easy method of removing any prostaglandin B_1 formed during storage of the intermediate. However, it was essential to elute the intermediate from the silica immediately after development of the plate to avoid fresh formation of prostaglandin B_1.

CONCLUSIONS AND DISCUSSION

The molecular ions in the mass spectra of both the methyl ester and the methyl ester-methoxime of the intermediate coincide with those of the corresponding derivatives of prostaglandins A_1 and B_1. The intermediate is thus an isomer of prostaglandins A_1 and B_1. The most probable alternative structures for the intermediate are depicted in Fig. 6. Compounds I and II are conjugated enols, whereas compound III has the 9-oxo function unconjugated from the diene grouping.

The ultraviolet spectroscopy data on the intermediate strongly support structure III. The spectrum of the intermediate is very similar to that of cholest-3,5-diene (λ_{max}^{MeOH} = 235 nm, with weaker bands at 228 and 243 nm; ϵ_{max}^{MeOH} = 19,700). Menthadiene (2-cyclohex-1-enylpropene), like compound III, is a semicyclic diene. The former has an extinction maximum at 235 nm in methanol (9), which is in close agreement with that found for the intermediate. Further evidence that the 234-nm chromophore of the intermediate derives from a conjugated diene grouping is the absence of a red shift upon changing the solvent from hexane to water. A shift of the absorption maximum to a higher wavelength with increased solvent polarity is characteristic

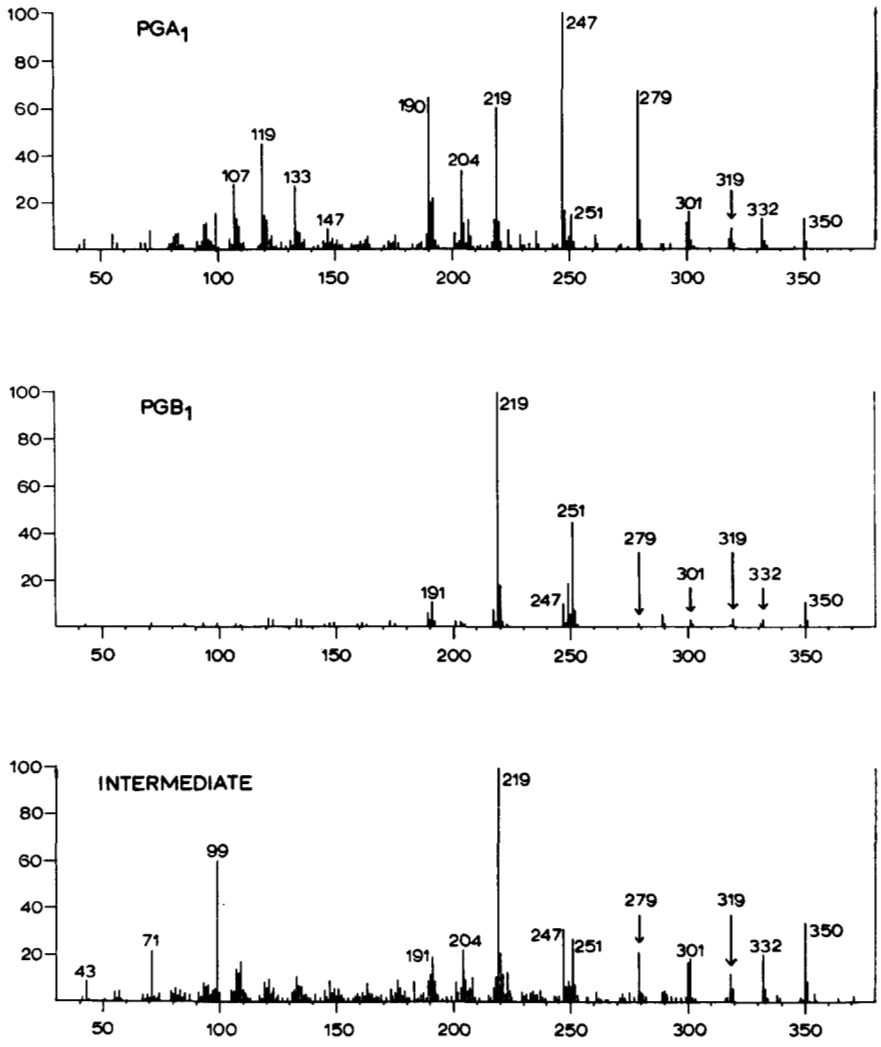

FIG. 5. Mass spectra of the methyl esters of prostaglandins A_1 and B_1 and the intermediate compound. Ordinate, percentage abundance; abscissa, m/e value.

of conjugated ketones (cf. prostaglandins A_1 and B_1) but not of conjugated dienes.

Compounds II and III are keto–enol tautomers. It is, however, unlikely that the intermediate is composed either entirely or to any appreciable extent of compound III, since the application of Woodward's rules (10) to the triene structure produces a value for λ_{max} in excess of 270 nm.

The formation of the methoxime derivative of the intermediate produces very little change in its ultraviolet spectrum, an observation which again supports the structure present in compound III since the diene grouping will be unconjugated from the methoxime grouping. It is difficult to envisage how compound I could react with methoxylamine without reverting to prostaglandin A_1 and forming the corresponding methoxime.

The value of 21,000, obtained from the molar extinction coefficient of the intermediate, would suggest that the diene grouping in the intermediate exists in the *trans*oid as opposed to the *cis*oid configuration.

The instability of the intermediate in weakly alkaline solution is in accord with the proposed structure (III). The loss of the tertiary C-8 proton in the 9-oxo-prostaglandins is facile, as evidenced by the ready interconversion of prostaglandin E_1 (Fig. 1) to the epimeric 8-iso prostaglandin E_1 in ethanolic potassium acetate at room temperature (11). The C-8 proton in compound III will be even more labile as it is activated by both the 9-oxo function and the 11,13-diene grouping. Hence, the enolization reaction shown in Fig. 6 will readily occur with the resultant formation of the stable dienone system of prostaglandin B_1.

The major peaks above m/e 210 in the mass spectra of the methyl ester derivatives of prostaglandins A_1 and B_1 and the intermediate isomer can be attributed to four fission processes (8, 12). Thus, the peaks at m/e 332 [M − 18], 319 [M − 31], and 301 [M − (31 + 18)]

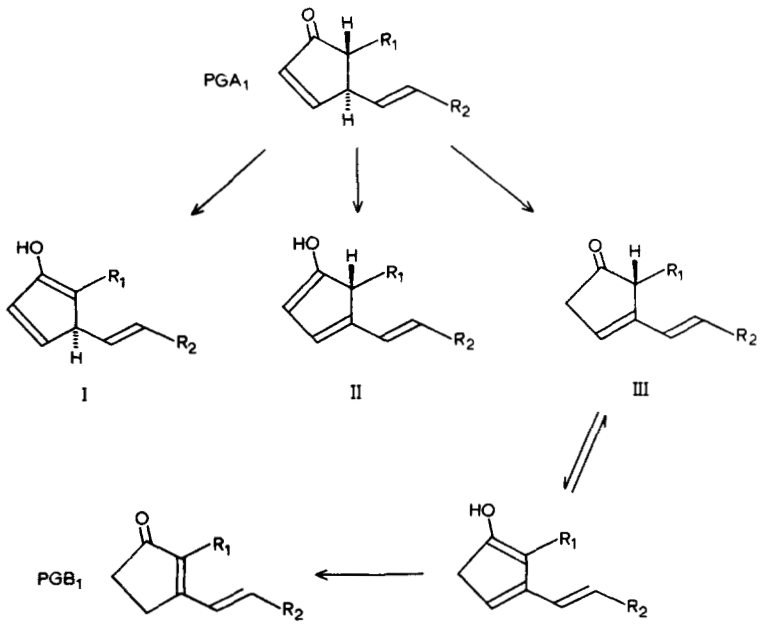

FIG. 6. Possible structures (I, II, and III) of the intermediate isomer resulting from the interaction of prostaglandin A_1 and prostaglandin isomerase. The base-catalyzed conversion of compound III to prostaglandin B_1 is also shown. $R_1 = (CH_2)_6COOH$ and $R_2 = CHOH(CH_2)_4CH_3$.

result from the loss of a molecule of water and the ester methoxyl. Elimination of the terminal 5-carbon unit in the ω side chain gives rise to the peak at *m/e* 279 (M − 71). In ω-homo prostaglandin A_1 a prominent peak also occurs at *m/e* 279 and corresponds to the loss of C_6H_{13} from the molecular ion (*m/e* 364). The peak at *m/e* 251 [M − (71 + 28)] is attributed to the combined loss of C_5H_{11} and CO from the cyclopentanone ring. The latter two peaks are always accompanied by larger peaks 32 mass units lower in the spectrum. In prostaglandins A_1 and B_1 these occur at *m/e* 247 [M − (71 + 32)] and 219 [M − (71 + 28 + 32)]. The elimination of CH_3OH as a neutral molecule accounts for this loss of 32 mass units (8). It has been found that in prostaglandins of the B series the peaks at M − (71 + 28 + 32) and M − (71 + 28) are always much greater than the corresponding peaks 28 mass units higher. In prostaglandins of the A series, however, the relative heights of these peaks are reversed. This situation may reflect the greater ease with which a molecule of carbon monoxide is lost from prostaglandins with the B ring structure as opposed to prostaglandins with the A ring structure. In this respect the spectrum of the intermediate isomer is similar to that of prostaglandin B_1.

In the spectrum of prostaglandin A_1 methyl ester, the major peaks at *m/e* values below 210 have their counterparts in the spectra of the three other prostaglandin A compounds examined (Table 2) and are thus indicative of a characteristic mode of fragmentation of the prostaglandin A skeleton. The proposed fragmentations resulting in the *m/e* 190 peak in prostaglandin A_1 methyl ester are shown in Fig. 7. The following data support this breakdown pattern: (*a*) prominent peaks at *m/e* 190 are present in the spectra of prostaglandins with altered α side chains, namely prostaglandins A_2 and α-nor A_1; (*b*) the *m/e* 190 peak in the spectrum of prostaglandin A_2 becomes the base peak, since fission is favored β to the 5,6 double bond; (*c*) in ω-homo prostaglandin A_1 the peak at *m/e* 190 is shifted to *m/e* 204. The *m/e* 147 [M − (142 + 18 + 43)], 133 [M − (142 + 18 + 57)], and 119 [M − (142 + 18 + 71)] peaks can all arise from additional fission processes in the ω side chain. The *m/e* 119 peak in prostaglandin A_1 methyl ester has a counterpart at *m/e* 148 (base peak) in the methyl ester-methoxime derivative.

The loss of the complete α side chain together with a molecule of water is not a favorable fission process in either the prostaglandin B derivatives (due to the position of the 8,12 double bond) or the intermediate derivatives.

A group of three prominent peaks at *m/e* 99, 71, and 43 occurs in both the methyl ester and the methyl ester-methoxime derivatives of the intermediate isomer. A metastable peak is also present in both spectra at *m/e* 51.0. This would suggest that the *m/e* 71 peak derives from the *m/e* 99 peak by the elimination of a neutral fragment, since m^* calculated $= \frac{(71)^2}{99} = 50.92$. The fragment ion at *m/e* 99 cannot be accounted for in terms of a simple fission process, and it has not been possible to formulate a satisfactory rearrangement. These peaks

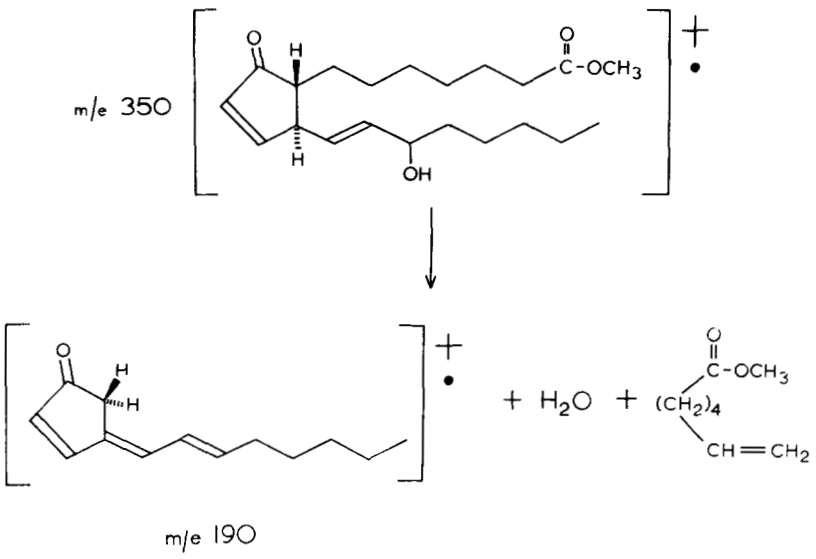

FIG. 7. Proposed mode of fragmentation of prostaglandin A_1 methyl ester resulting in the formation of the ion at m/e 190 [M − (142 + 18)].

do not, therefore, shed any light on the structure of the intermediate isomer.

Information from nuclear magnetic resonance and infrared studies is required for the conclusive elucidation of the structure of the intermediate isomer. The infrared data obtained in this investigation do not provide any definitive evidence. This is because the resolution in the carbonyl stretching regions is insufficient due to the small amount of substance in the disc (0.5–1 mg), the unsatisfactory method of incorporation of the substance into the disc, and the isomerization of the intermediate in the disc to prostaglandin B_1. It is hoped that future work may provide enough of the intermediate for the recording of a "solution" infrared spectrum and also for nuclear magnetic resonance measurements.

Although the structure of the intermediate isomer has not been unequivocally established in the present investigation, it is felt that the available evidence points strongly to the 9-oxo-11,13-diene structure (compound III in Fig. 6).

Preliminary investigations into the enzymatic isomerization of prostaglandin A_1 to B_1 showed that the rate of prostaglandin B_1 formation and, apparently, enzyme activity were optimal at pH 8.5 and relatively slow at pH 7 (4). From the present studies it is clear that these pH activity measurements are a reflection of the chemical stability of the intermediate isomer and not a true estimate of the pH dependence of the isomerase.

Prostaglandins of the E, A, and B series can be isolated in large amounts from human seminal plasma. Hamberg and Samuelsson (13) have presented evidence to show that the majority of extracted prostaglandin A was originally present in the seminal plasma and was probably formed enzymatically from prostaglandin E. The origin of the prostaglandins B, however, is in some doubt. They could be formed nonenzymatically from prostaglandins A during storage in the male reproductive system or during the isolation procedure. Alternatively, they may arise from the corresponding intermediate isomers during storage in vivo or during isolation. The presence of the 9-oxo-11,13-diene isomers of the prostaglandins A in human seminal plasma has not been demonstrated, and in view of the low stability of these compounds this is hardly surprising. However, Hamberg and Samuelsson (13) have reported that human seminal plasma contains a small amount of a substance that cochromatographs with the prostaglandins A and B on a silicic acid column but is slightly less polar than the latter compounds on reversed-phase partition chromatography with solvent system F55. The substance exhibits ultraviolet absorption at 278 nm only after treatment with dilute alkali. Characterization of the substance was not achieved. These properties would suggest that the substance could be the 9-oxo-11,13-diene isomer of a prostaglandin A; the present investigation has provided sufficient data on the latter compounds to prove or disprove the assumption in future studies.

Biological studies on the intermediate isomer in this laboratory have shown that it is a more potent vasodilator than its precursor, prostaglandin A_1, and, like prostaglandin A_1 (14, 15), passes through the pulmonary circulation without loss of activity. This is in contrast to the prostaglandins E, which are efficiently inactivated by the lung in vivo (16). It has been speculated that the prostaglandins A may act as circulating hormones, controlling, for example, renal sodium excretion or peripheral vascular resistance (17, 18). If this is the case, then the activity of blood-borne prostaglandin A may be

augmented after its conversion to the 9-oxo-11,13-diene isomer by prostaglandin isomerase.

Studies are in progress to assess the significance of the enzymatic isomerization of the prostaglandins A in vivo and also to determine the mechanism of action and properties of prostaglandin isomerase.

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