Pergamon

Bioorganic & Medicinal Chemistry, Vol. 3, No. 4, pp. 459–469, 1995

0968-0896/95 $9.50 + .00

0968-0896(95)00037-2

FTIR Spectral Study of Intramolecular Hydrogen Bonding in E-Type of 15-Keto-prostaglandins in Dilute CCl_4 Solution: Structure–Activity Relationships

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Abstract—FTIR spectra of 15-keto-prostaglandin (PG)E_2 (**1**), 15-keto-PGE_1 (**2**), 13,14-dihydro-15-keto-PGE_2 (**3**), 13,14-dihydro-15-keto-PGE_1 (**4**), and their related compounds were measured in dilute tetrachloromethane solution in order to examine the structure–activity relationships for the 5,6-*cis*-double bond of the α-side chain and the 13,14-*trans*-double bond and the 15-hydroxyl group of the ω-side chain in PGE_2, PGD_2, and $PGF_{2\alpha}$. The spectra were subjected to curve analysis to separate overlapping absorption bands. For compounds **1–4**, an intramolecular hydrogen bond involving a 15-membered ring similar to that observed for PGE_2, PGD_2, and $PGF_{2\alpha}$ was found between the carboxyl and 15-carbonyl groups. The percentages (ρ) of the intramolecular hydrogen-bonded molecules with the 15-membered rings in **1–4** and PGE_2 were compared with the known binding activities of PGs for various PG receptors, and we found that these activities decrease as the ρ values decrease. These results strongly supported our hypothesis that, in PGs, the conformation with the 15-membered ring formed by the intramolecular hydrogen bonds between the carboxyl group of the α-side chain and the 15-hydroxyl group of the ω-side chain is a precursory conformation of the active one.

Introduction

Thromboxane (TX)A_2,[1] prostaglandin (PG)E_2,[2] PGD_2,[3] and $PGF_{2\alpha}$ [4] which have the same α- and ω-side chains as shown in Figure 1 possess various physiological activities at very low levels.[5] Toda *et al.* reported that a TXA_2 receptor agonist ($sTXA_2$)[6] and PGE_2, PGD_2, and $PGF_{2\alpha}$ share the same receptor site responsible for vascular contraction,[7] and that PGD_2 shares the mechanism underlying arterial contraction with PGE_2 and $PGF_{2\alpha}$.[8] Assuming that the binding site is in a hole in protein, we have been interested in the conformations of the α- and ω-side chains in these compounds in CCl_4, where the environment is similar to that of its binding site as reported previously.[9,10] On the basis of analysis of FTIR spectra of a TXA_2 receptor agonist (U-46619)[11] and of PGE_2, PGD_2, and $PGF_{2\alpha}$ in dilute CCl_4 solution, we were able to establish the conformation (A) with a 15-membered ring formed by the intramolecular hydrogen bond (I) between the carboxyl and 15-hydroxyl groups as shown in Figure 2.[9] An active conformation of these compounds was anticipated to differ from the intramolecular hydrogen-bonded one because the carboxyl group appears to be of importance in the exhibition of the physiological activity. However, the percentage of the intramolecular hydrogen-bonded molecules with the 15-membered ring in CCl_4 showed a high value of *ca* 80% for these compounds.[9] Therefore, in order to elucidate the structure–activity relationships related to the side chains for these compounds, we hypothesized that even if the active conformation is of the nonintramolecular hydrogen-bonded one (B) (Fig. 2), the conformation (B) would be analogous to the conformation (A) formed by the hydrogen bond (I), where the hydrogen bond would play an important role in the formation of a conformation suitable for the binding site in the receptor.[9]

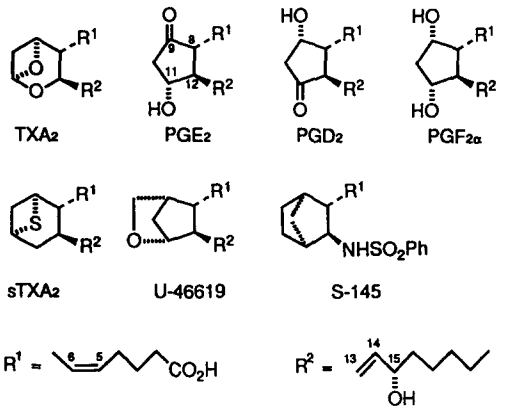


Figure 1. TXA_2, PGE_2, PGD_2, $PGF_{2\alpha}$, and TXA_2 receptor agonists ($sTXA_2$ and U-46619) and its antagonist (S-145).

For six TXA_2 receptor antagonists including S-145,[12] we found that the intramolecular hydrogen bonds involving a large-membered ring of more than 11 are formed between the carboxyl group of the α-side chain and the functional groups in dilute CCl_4 solution, and we reported a geometrical resemblance of the side chain and the functional group among all of these compounds.[13,14] In addition, we revealed that the percentage (ρ) of the intramolecular hydrogen-bonded molecules and TXA_2 receptor antagonistic potency in

the S-145-type compounds which form the optimum 12-membered ring decrease as the degree of freedom in the motions of the carboxyl and (phenylsulfonyl)amino groups increases.[15] According to the above hypothesis, we predicted that, for prostaglandins (PGs), an increase in the degree of freedom in the motions of the α- and ω-side chains results in a decrease of both the ρ value and the binding ability to the receptor. If this prediction can be clarified, it would not only be of considerable interest physiologically, but of the greatest importance in confirming our hypothesis.

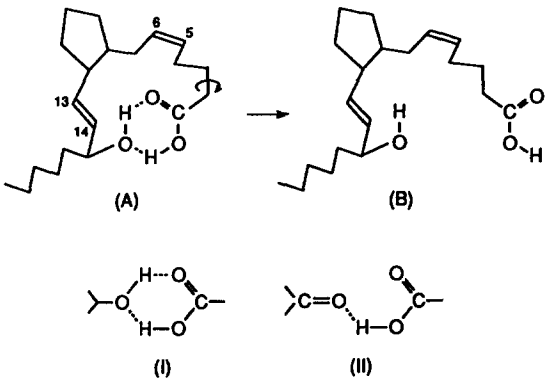


Figure 2. Conformations of the intramolecular hydrogen-bonded molecule (A) and its cleaved molecule (B). The functional groups on the cyclopentane ring were omitted.

Numerous investigators reported on the effects of 5,6-*cis*- and 13,14-*trans*-double bonds and the 15-hydroxyl group on specific binding activities of PGE_2, PGD_2, and $PGF_{2\alpha}$ and their analogues for their receptor sites.[16-20] We directed our attention to the relationships between the specific binding activity and the ρ value in PGs used in these studies. However, it is extremely difficult to obtain ρ values of the same types of PGs which have the 15-hydroxyl group because $PGF_{2\alpha}$ is not very soluble in CCl_4, PGE_1 is only slightly soluble,[9] and the solubility of PGD_1 is surmised to be similar to that of PGE_1. These solubilities usually increase as the number of hydroxyl groups decreases. Recently, we found that the intramolecular hydrogen bond (II) involving a 14-membered ring in an analogous compound of S-145 is formed between the carboxyl group and the carbonyl bond of the amido group.[21] From these findings, 15-keto-PGEs **1–4**, which are converted from the 15-hydroxyl groups to the carbonyl groups, and their related compounds **5–7** were chosen as model compounds (Fig. 3) because the ρ value which is attributable to the functional groups on the cyclopentane ring of PGE_2 is the smallest in comparison with those of PGD_2 and $PGF_{2\alpha}$.[9] The FTIR spectra of **1–11** were measured in dilute CCl_4 solution and subjected to curve analysis in order to separate overlapping absorption bands. For **1–4**, the intramolecular hydrogen bond (II) involving the 15-membered ring similar to those observed for PGE_2, PGD_2, and $PGF_{2\alpha}$ was found between the carboxyl and 15-carbonyl groups,[9] and their ρ values were estimated. No study of systematically conformational analysis using physicochemical quantities, except for the ρ value, has been reported on the active conformation of PGs. On the basis of these results, we examined the structure–activity relationships of the α- and ω-side chains for PGs and the validity of our hypothesis.

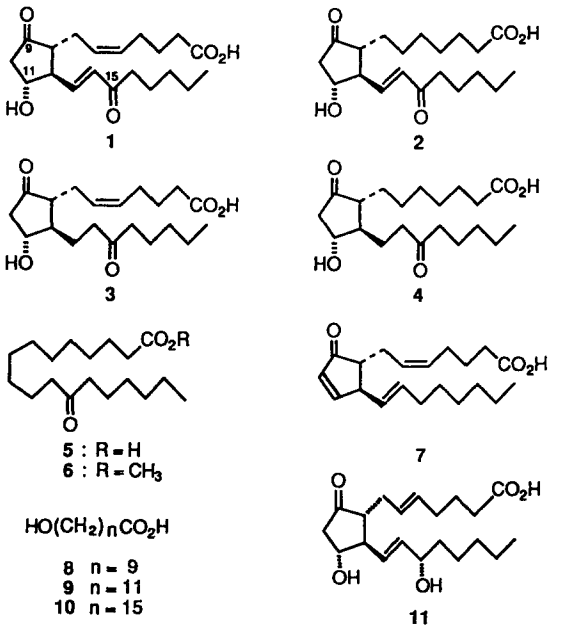


Figure 3. E type of 15-keto-PGs, **1–4**, and their related compounds **5–11**.

Results and Discussion

In general, the formation of the intramolecular O-H•••O=C bond causes a shift of the ν_{OH} and $\nu_{C=O}$ bands to lower wavenumbers.[23,24] When the intramolecular hydrogen bonds of the (I) and (II) types are formed between the carboxyl and hydroxy groups and between the carboxyl and carbonyl groups, respectively, not only the ν_{OH} and $\nu_{C=O}$ bands of the former carboxyl group but also those of the latter one shift to lower wavenumbers in spite of the fact that the C=O bond of the latter carboxyl group does not form the hydrogen bond.[9,13,21] The spectral parameters obtained for **1–11** are shown in Table 1 together with the assignments of the peaks and the *N*, σ, and ρ values.

Intramolecular hydrogen bonds between functional groups on cyclopentane ring and carboxyl group

As reported previously, PGE_2 and 15-deoxy-PGE_2, which was deoxidized, do not form the intramolecular hydrogen bond (II) between the 9-carbonyl group on the cyclopentane ring and the carboxyl group of the α-side chain.[9] In addition, compound **7** does not form a similar hydrogen bond as shown below. These findings indicate that the intramolecular hydrogen bond (II) in **1** and **3** is not formed between the 9-carbonyl and carboxyl groups. For **1** and **3**, the ε values of 620 and 615 mol^{-1} dm^3 cm^{-1} were observed for the free $\nu_{C=O}$ band for the 9-carbonyl group, respectively. Actually, these values are identical with 618 mol^{-1} dm^3 cm^{-1} for PGE_2 and 614 mol^{-1} dm^3 cm^{-1} for 15-deoxy-PGE_2 within the experimental error

Table 1. FTIR spectral data[a] of compounds 1–11 in CCl_4

Compd.	Assign.[b]			ν_{OH} or $\nu_{C=O}$ /cm^{-1}	ε /mol^{-1} dm^{3}cm^{-1}	$\Delta\nu_{1/2}$ /cm^{-1}	A/10^{-8} cm^{2}s^{-1} molecule^{-1}	N[c] / %	σ[d] /%	ρ[e] /%	c[f] /10^{-5} mol dm^{-3}
1	CO_2H	ν_{OH}	F	3532.1	54.7	29.3	22.9	31	1	68	3.4640
			H[g]	*ca*.3230							
		$\nu_{C=O}$	F	1758.9	158.4	19.1	37.3				
			H	1742.8	295.6	12.3	44.8				
			H	1733.2	189.8	18.7	44.2				
	9-C=O	$\nu_{C=O}$	F	1751.5	620.3	11.1	86.5				
	15-C=O	$\nu_{C=O}$	F[h]	1702.4	74.4	22.8	22.1				
			F[i]	1685.0	109.6	14.0	19.2				
			H[h]	1667.0	44.5	14.9	7.54				
			H[i]	*j*							
2	CO_2H	ν_{OH}	F	3532.6	93.4	26.0	34.8	52	4	44	3.1605
			H[g]	*ca*.3230							
		$\nu_{C=O}$	F[h]	1758.8	265.8	19.6	67.2				
			H	1738.6	116.0	11.6	17.4				
			H	1731.3	127.7	17.4	28.8				
	9-C=O	$\nu_{C=O}$	F	1749.7	615.8	12.1	97.4				
	15-C=O	$\nu_{C=O}$	F[h]	1702.6	143.3	21.5	39.9				
			F[i]	1682.9	92.3	13.9	15.9				
			H[h]	1667.7	32.7	14.6	5.6				
			H[i]	*j*							
3	CO_2H	ν_{OH}	F	3533.6	95.9	29.2	39.2	54	5	41	3.3761
			H[g]	*ca*.3260							
		$\nu_{C=O}$	F	1758.4	272.4	18.8	65.9				
			H	1736.0	214.2	14.2	38.6				
	9-C=O	$\nu_{C=O}$	F	1746.9	614.7	12.9	100.0				
	15-C=O	$\nu_{C=O}$	F[k]	1717.6	220.8	16.2	45.4				
			H[i]	1704.3	176.8	19.2	42.4				
4	CO_2H	ν_{OH}	F	3533.8	115.4	24.6	39.5	65	8	27	3.5375
			H[g]	*ca*.3245							
		$\nu_{C=O}$	F	1758.7	323.5	19.5	83.0				
			H	1734.4	147.7	13.8	27.8				
	9-C=O	$\nu_{C=O}$	F	1746.0	620.2	12.1	103.4				
	15-C=O	$\nu_{C=O}$	F[k]	1715.6	275.7	15.8	53.5				
			H[i]	1703.6	129.8	17.8	28.7				
5	CO_2H	ν_{OH}	F	3532.9	139.8	22.0	39.3	78			3.6252
			H[g]	*ca*.3250							
		$\nu_{C=O}$	F	1758.7	390.5	19.3	98.8	(78)	12	10	
			H	1738.2	96.2	16.6	21.0				
			D[m]	1710.5	94.7	12.7	14.8				
	12-C=O	$\nu_{C=O}$	F	1716.0	331.1	15.5	63.4			(9)	
			H	1699.1	51.8	16.3	10.4				
6	CO_2CH_3	$\nu_{C=O}$	F	1741.7	574.3	15.0	117.1				3.4432
	12-C=O	$\nu_{C=O}$	F	1715.8	362.7	15.8	80.6				
7	CO_2H	ν_{OH}	F	3532.4	150.1	24.5	51.4	84			3.2406
		$\nu_{C=O}$	F	1758.2	429.9	19.3	108.7	(86)	12	2	
			D[m]	1710.5	102.8	12.7	17.9				
	9-C=O	$\nu_{C=O}$	F	1713.9	775.7	14.2	168.0				
8	CO_2H	ν_{OH}	F	3533.0	148.0	23.1	49.3	83			2.6347[n]
		$\nu_{C=O}$	F	1758.8	386.4	19.6	101.8	(77)	8	15	
			H	1735.1	64.5	20.2	15.7				
			D	1711.3	97.2	14.5	16.5		(12)		

Table 1. *Continued*

Compd.	Assign.[b]			ν_{OH} or $\nu_{C=O}$ /cm^{-1}	ε /mol^{-1} dm^{3}cm^{-1}	$\Delta\nu_{1/2}$ /cm^{-1}	$A/10^{-8}$ cm^{2}s^{-1} molecule^{-1}	N[c] / %	σ[d] /%	ρ[e] /%	c[f] /10^{-5} mol dm^{-3}
9	CO_2H	ν_{OH}	F	3532.3	118.8	23.8	38.9	67			3.6508[n]
		$\nu_{C=O}$	F	1758.6	338.6	19.6	87.8	(67)	9	24	
			H	1734.1	102.0	21.0	26.2				
			D	1711.1	97.0	15.4	20.1		(12)		
10	CO_2H	ν_{OH}	F	3532.6	109.2	23.6	36.6	61			4.3082
		$\nu_{C=O}$	F	1758.6	305.7	19.1	77.9	(61)	8	31	
			H	1733.9	123.8	21.9	33.1				
			D	1710.8	88.5	14.5	18.0		(11)		
11	CO_2H	ν_{OH}	F	3532.2	36.3	23.6	10.8	20			3.2797
			H[g]	*ca*.3350							
		$\nu_{C=O}$	F	1761.4	120.9	14.5	22.0	(24)	1	75	
			H	1734.5	154.0	18.9	38.0				
			H	1710.2	181.1	23.5	55.6				
	9-C=O	$\nu_{C=O}$	F	1748.8	611.8	12.9	101.8				

[a] ν, ε, $\Delta\nu_{1/2}$, and A are the frequency of stretching vibration band, the molar absorption coefficient, the band width at half-intensity, and the integrated intensity, respectively. These parameters of the ν_{OH} bands of the hydroxyl group are not given here.

[b] F, H, and D show non-hydrogen-bonded and intramolecular hydrogen-bonded molecules and dimers, respectively.

[c] Percentage (N) of non-hydrogen-bonded molecules. The N values of **1**–**4** were estimated using the ε value of the free ν_{OH} band of the carboxyl group and the equation [N = (ε/178.4)100] because the free $\nu_{C=O}$ band of the carboxyl group decreases and is overlapped by the $\nu_{C=O}$ band of the 9-carbonyl group, where 178.4 is the ε value of 100% free ν_{OH} band of lauric acid.[13] Therefore, the parameters of the free $\nu_{C=O}$ band of the carboxyl group were calculated from the N value estimated and the parameters of lauric acid.[13] The peak separation of the spectrum of these compounds in the region of the $\nu_{C=O}$ bands was carried out while the parameters of this free $\nu_{C=O}$ band are fixed. The N values in parentheses were estimated using the ε value of the free $\nu_{C=O}$ band of the carboxyl group and the equation [N = (ε/501.9)100], where 501.9 is the ε value of 100% free $\nu_{C=O}$ band of lauric acid.[13]

[d] Percentage (σ) of dimers: the value was approximately estimated using the equations $\log c_f = 0.245\sigma_o^{1/2} - 5.492$ and $\sigma = \sigma_o N/100$, where c_f is the concentration of non-hydrogen-bonded molecules ($c_f = cN/100$) and σ_o is the percentage of dimers at c_f.[14,15] The σ values in parentheses were estimated using the ε value of the dimer $\nu_{C=O}$ band of the carboxyl group and the equation [σ = (ε/822.6)100], where 822.6 is the true ε value per $\nu_{C=O}$ band of dimer in lauric acid.[13]

[e] Percentage (ρ) of intramolecular hydrogen-bonded molecules, $\rho = 100-(N+\sigma)$. The ρ value in parentheses was estimated using the ε value of the free $\nu_{C=O}$ band of the 12-carbonyl group and the equation ρ = 100–(ε/362.7)100, where 362.7 is the ε value of the $\nu_{C=O}$ band of the 12-carbonyl group of **6** which is incapable of hydrogen bonding.

[f] Concentration.

[g] The exact parameters could not be obtained because the band was very weak and broad.

[h] The (III) type.[25]

[i] The (IV) type.[25]

[j] The parameters were not obtained because the band was overlapped by solvent absorption.

[k] The band would be overlapped by the dimer $\nu_{C=O}$ band at *ca* 1711 cm^{-1}.[13]

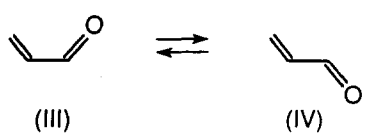
[l] The band would be overlapped by the intramolecular hydrogen-bonded $\nu_{C=O}$ band formed between the 15-carbonyl and 11-hydroxyl groups.

[m] The parameters were estimated from the σ value[d] and the parameters of the dimer $\nu_{C=O}$ band in lauric acid[13] because the band is weak and is overlapped by the $\nu_{C=O}$ band of the 12- or 9-carbonyl group.

[n] The average of total A values of the $\nu_{C=O}$ bands for the carboxyl group observed for PGs was 134 × 10^{-8} cm^2 s^{-1} molecule^{-1}.[9] The c values of **8** and **9** were estimated from this value because these compounds could not be completely dissolved in CCl_4.

limit. On the other band, the ε values of 616 and 620 mol^{-1} dm^3 cm^{-1} were observed for the same $\nu_{C=O}$ band in the E_1-type of **2** and **4** of which the 5,6-*cis*-double bonds were saturated, respectively. These values are identical with those of the above compounds. From this result, it was presumed that **2** and **4** do not form the intramolecular hydrogen bond (II) between the 9-carbonyl and carboxyl groups.

However, 15-deoxy-PGE_2 forms the intramolecular hydrogen bond (I) between the 11-hydroxyl group on the cyclopentane ring and carboxyl group and its ρ value is 8%.[9] As the ρ values of **1**–**4** are 3.4–8.5 times higher than that of 15-deoxy-PGE_2, it is presumed that their carboxyl groups are mainly connected to the 15-carbonyl group of the ω-side chain by intramolecular hydrogen bonding. These major hydrogen bonds (II) would probably suppress the formation of a minor hydrogen bond (I) between the 11-hydroxyl and carboxyl groups. For minor hydrogen bonding, the ρ values of **1**–**4** cannot be estimated due to overlapping of the major $\nu_{C=O}$ bands. These values were ignored in this case because the effects mentioned in the Introduction were evaluated on the basis of the differences between the ρ values for the E-type of PGs (PGEs). The *s-cis*-(III) and *s-trans*-conformers (IV) coexist at equilibrium in **1** and **2** which have an α,β-unsaturated ketone on the ω-side chain.[25] The spectral changes in the ν_{OH} and $\nu_{C=O}$ bands, except for the $\nu_{C=O}$ band of the 9-carbonyl group, will be described below.



(III) (IV)

15-Keto-PGE$_2$ (1) and 15-keto-PGE$_1$ (2)

The intensities of the free ν_{OH} bands at 3532 cm^{-1} for the carboxyl group in **1** and at 3533 cm^{-1} in **2** decreased markedly and new broad bands appeared at *ca* 3230 cm^{-1}. As shown in Figure 4, the intensities of the free $\nu_{C=O}$ bands at 1759 cm^{-1} for the carboxyl groups in **1** and **2** also decreased and two new bands appeared at 1743 and 1733 cm^{-1}, and 1739 and 1731 cm^{-1}, respectively, suggesting that an equilibrium exists between two conformers which are attributable to the α,β-unsaturated ketone moiety. Correspondingly, the intensities of the free $\nu_{C=O}$ bands at 1702 and 1685 cm^{-1} for the 15-carbonyl groups of the (III) and (IV) types in **1** and at 1703 and 1683 cm^{-1} in **2**, respectively, decreased and new bands of the (III) type appeared at 1667 and 1668 cm^{-1}, respectively. However, new bands of the (IV) type could not be observed because of solvent absorptions. These spectral changes of **1** are larger than those of **2**. From these findings, it is clear that, for these compounds, the intramolecular hydrogen bonds (II) involving the 15-membered ring similar to (A) are formed between the 15-carbonyl and carboxyl groups. The ρ values of **1** and **2** were estimated to be 68 and 44%, respectively.

13,14-Dihydro-15-keto-PGE$_2$ (3) and 13,14-dihydro-15-keto-PGE$_1$ (4)

For **3** and **4**, the intensities of the free ν_{OH} bands at 3534 cm^{-1} for the carboxyl group moderately decreased and new broad bands appeared at *ca* 3260 and *ca* 3245 cm^{-1}, respectively. As shown in Figure 5, the intensities of the free $\nu_{C=O}$ bands at 1758 cm^{-1} for the carboxyl group in **3** and at 1759 cm^{-1} in **4** also decreased and new bands appeared at 1736 and 1734 cm^{-1}, respectively. Correspondingly, the intensities of the free $\nu_{C=O}$ bands at 1718 cm^{-1} for the 15-carbonyl group in **3** and at 1716 cm^{-1} in **4** decreased and new bands appeared at 1704 cm^{-1}. These spectral changes of **3** are

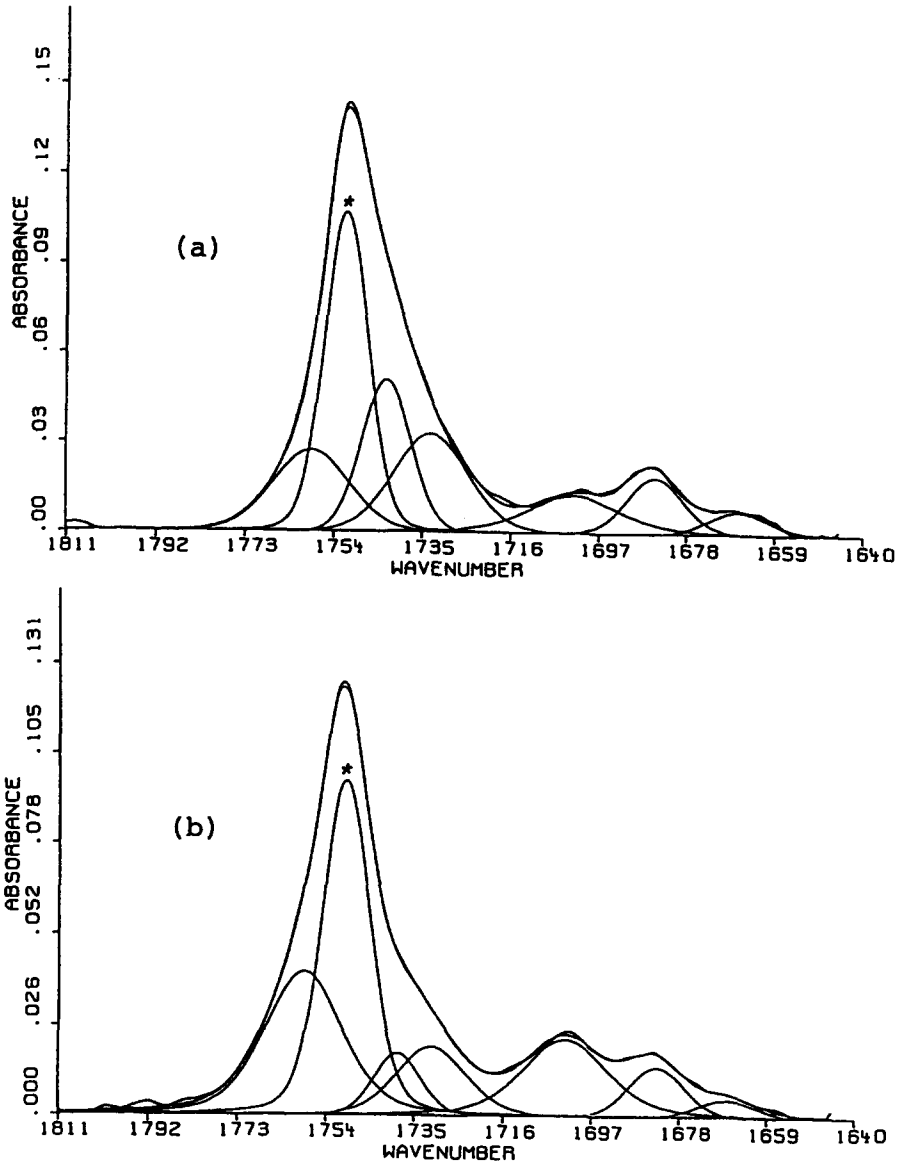


Figure 4. FTIR spectra of **1** (upper) and **2** (lower) in CCl_4 and the results of peak separations of their spectra. Spectra were obtained with a 5.0-cm cell: (a) **1**, 3.4640 × 10^{-5} mol dm^{-3} and (b) **2**, 3.1605 × 10^{-5} mol dm^{-3}.* The free $\nu_{C=O}$ band of the 9-carbonyl group.

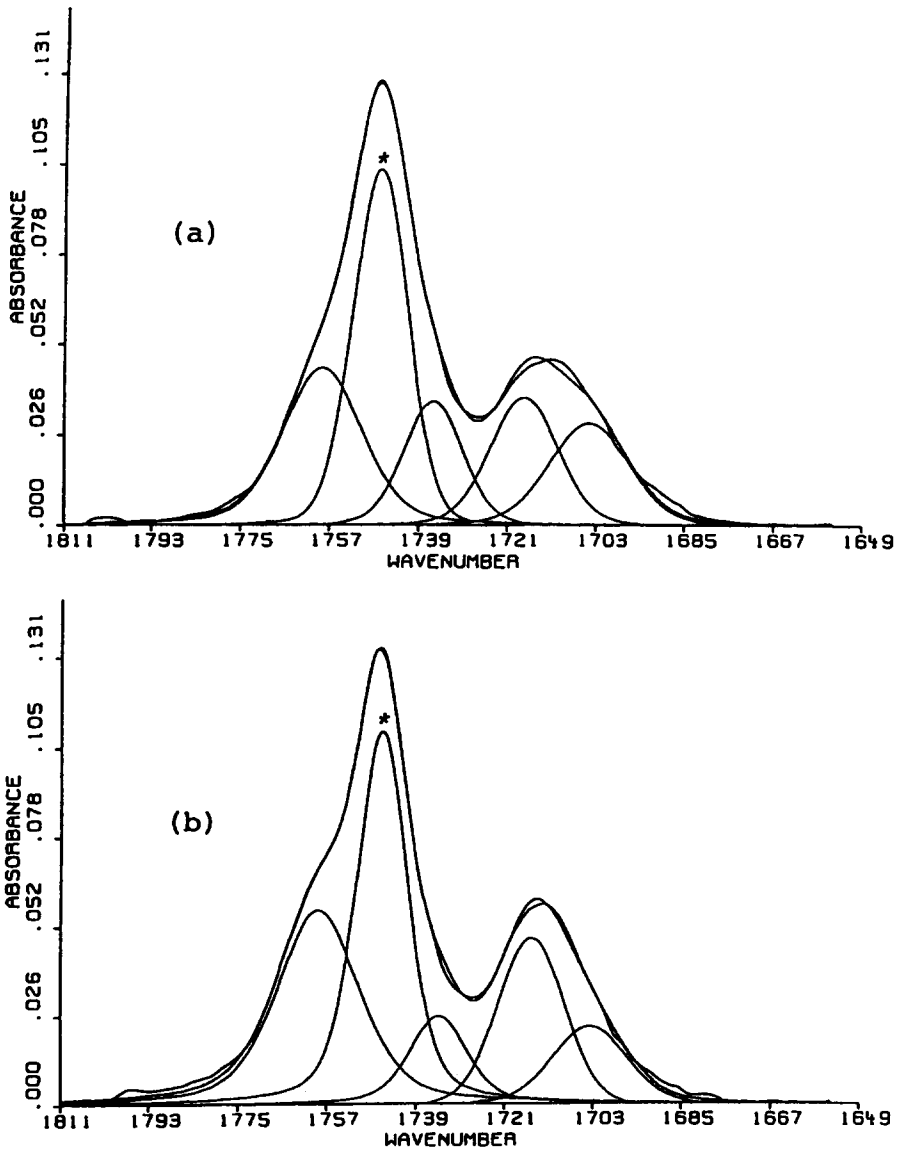


Figure 5. FTIR spectra of **3** (upper) and **4** (lower) in CCl_4 and the results of peak separations of their spectra. Spectra were obtained with a 5.0-cm cell: (a) **3**, 3.3761×10^{-5} mol dm^{-3} and (b) **4**, 3.5375×10^{-5} mol dm^{-3}.* The free $\nu_{C=O}$ band of the 9-carbonyl group.

larger than those of **4**. From these results, it is apparent that, in these compounds, the intramolecular hydrogen bonds (II) involving the 15-membered ring similar to (A) are formed between the 15-carbonyl and carboxyl groups. The ρ values of **3** and **4** were estimated to be 41 and 27%, respectively.

In **3** and **4**, additional intramolecular hydrogen bond can be formed between the 11-hydroxyl and 15-carbonyl groups. Compounds **3** and **4** gave the hydrogen-bonded ν_{OH} bands at 3497 and 3499 cm^{-1}, respectively. Although these hydrogen bonds slightly inhibit the formation of the hydrogen bond (II) between the 15-carbonyl and carboxyl groups, these contributions to the ρ value are neglected because their bands are very weak.[26]

12-Oxooctadecanoic acid (5) and 15-deoxy-PGA$_2$ (7)

For **5**, the intensities of the free ν_{OH} band at 3533 cm^{-1} and the free $\nu_{C=O}$ band at 1759 cm^{-1} for the carboxyl group partly decreased, and the new broad band and the new band appeared at *ca* 3250 and 1738 cm^{-1}, respectively. Correspondingly, the intensity of the free $\nu_{C=O}$ band at 1716 cm^{-1} for the 12-carbonyl group decreased and a new band appeared at 1699 cm^{-1} as shown in Figure 6. These findings indicate that the intramolecular hydrogen bond (II) involving a 15-membered ring similar to (A) is formed between these groups. The ρ value of **5** was estimated to be 10%, which is very close to the 9% estimated using the ε value of the free $\nu_{C=O}$ band for the 12-carbonyl group in **6**.

In **7** as shown in Figure 6, the free $\nu_{C=O}$ bands of the 9-carbonyl and carboxyl groups do not cause the lower wavenumber shifts which are attributable to the intramolecular hydrogen bond. The ρ value of **7** was estimated to be 2% which is within the experimental error limit. This indicates that **7** does not form the intramolecular hydrogen bond (II) between the 9-carbonyl and carboxyl groups.

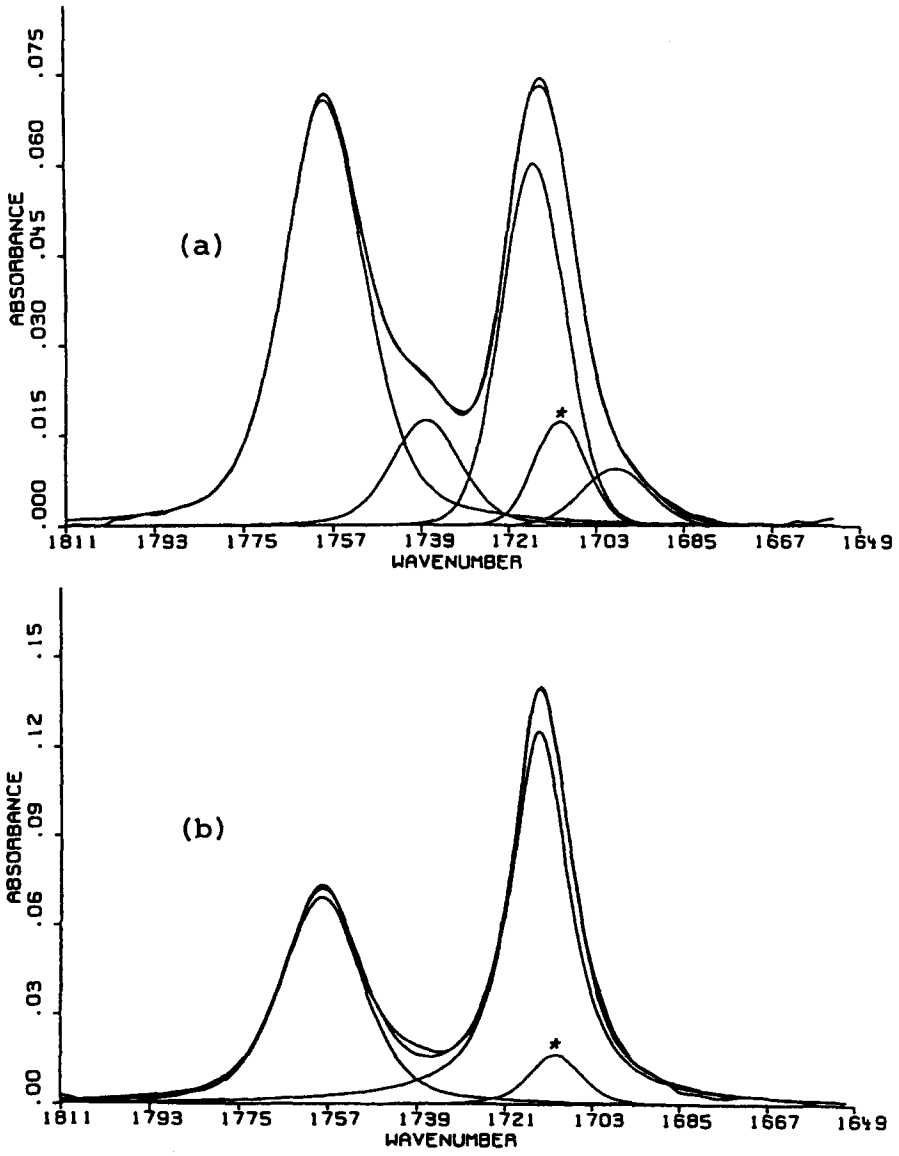


Figure 6. FTIR spectra of **5** (upper) and **7** (lower) in CCl_4 and the results of peak separations of their spectra. Spectra were obtained with a 5.0-cm cell: (a) **5**, 3.6252×10^{-5} mol dm^{-3} and (b) **7**, 3.2406×10^{-5} mol dm^{-3}.* The dimer $\nu_{C=O}$ band of the carboxyl groups (see Table 1, footnote *m*).

As predicted, the ρ values become larger in the order of **5**, **4**, **3**≈**2**, and **1**, although the 15-membered ring is formed in these compounds. This indicates that the degree of freedom in the motions of the carboxyl and 15- or 12-carbonyl groups becomes smaller in this order. Thus the following examination was carried out by analysis of the ρ values of **1**–**4**, PGE_2 of which the ρ value is larger than that of **1** due to the intramolecular hydrogen bond (I) of the 15-hydroxyl group, and 15-deoxy-PGE_2 which does not have this group.[27]

Structure–activity relationships

In order to elucidate the effects of saturating the 5,6-*cis* and/or 13,14-*trans*-double bonds or converting the 15-hydroxyl into a carbonyl group or a hydrogen atom in PGs, we compared the decreases (Δρ) of the ρ values which are attributable to these effects with the corresponding changes on the binding activities of PGs for PG binding sites in various receptors:[16–20] the binding site for PGE_2 in fetal calf adrenals,[16a] that for PGE_1 in rhesus myometrium,[17] that for PGD_2 in rat brain synaptic membrane,[18] that for $PGF_{2\alpha}$ in bovine and ovine corpora lutea,[19] and the primary PG(PPG) binding site in rat vascular smooth muscle cells.[20] The changes of these binding activities are given by the ratios of the 50% inhibitory doses (ID_{50}) or concentrations (IC_{50}) of PGs for the bindings of labeled PGE_2 and PGD_2 in the corresponding binding sites and of labeled $PGF_{2\alpha}$ and PGE_1 in the PPG one and the ratios of the dissociation constants (K_d) for the bindings of PGs to the $PGF_{2\alpha}$ binding site and the reciprocals of the ratio of the relative binding affinities (BA) of PGs for the PGE_1 binding site. These values are listed in Table 2 together with the corresponding Δρ values. To avoid any influence from the functional groups on the cyclopentane ring, we adopted the ratios of the ID_{50}, IC_{50}, K_d, or BA values for the same type of PGs. The qualitative findings (1)–(5) were obtained on the basis of the Table 2 results as follows.

Table 2. Effects of functional groups on the ρ value of prostaglandins (PGs) and on 50% inhibitory dose (ID_{50}), relative binding affinity (BA), 50% inhibitory concentration (IC_{50}), and dissociation constant (K_d) for binding of PGs to various kinds of PG receptors

No.	Effect[a]	Prostaglandin X→Y		Δρ/% [b]	PGE_2[c] $ID_{50}(X)/ID_{50}(Y)$	PGE_1[d] BA(Y)/BA(X)	PGD_2[e] $IC_{50}(X)/IC_{50}(Y)$	$PGF_{2\alpha}$[f] $K_d(X)/K_d(Y)$	PPG[g] $IC_{50}(X)/IC_{50}(Y)$
(1)	C(5)=C(6)	1	2	–24					
		PGE_2	PGE_1		1/1.4	1/2	1/1.3	1/16 (1/13)	1 (1/1.4)
		PGD_2	PGD_1				1/28		
		$PGF_{2\alpha}$	$PGF_{1\alpha}$					1/40 (1/20)	
(2)	C(13)=C(14)	1	3	–27					
		$PGF_{2\alpha}$	13,14-dihydro-$PGF_{2\alpha}$					1/4 (1/2)	1/36 (1/14)
		15-keto-$PGF_{2\alpha}$	13,14-dihydro-15-keto-$PGF_{2\alpha}$					1/2	1/3 (1/2)
		2	4	–17		1			
		PGE_1	13,14-dihydro-PGE_1			1/5			
(3)	C(5)=C(6) and	1	4	–41					
	C(13)=C(14)	PGE_2	13,14-dihydro-PGE_1			1/10			
(4)	15-OH / 15-C=O	PGE_2	1	–14					
		PGE_1	2			1/56			
		13,14-dihydro-PGE_1	4			1/12			
		$PGF_{2\alpha}$	15-keto-$PGF_{2\alpha}$					1/180	1/115 (1/91)
		13,14-dihydro-$PGF_{2\alpha}$	13,14-dihydro-15-keto-$PGF_{2\alpha}$					1/70 (1/48)	1/8 (1/12)
(5)	15-OH / 15-H	PGE_2	15-deoxy-PGE_2	–74					
		$PGF_{2\alpha}$	15-deoxy-$PGF_{2\alpha}$	–47[h]					1/418 (1/91)

[a] C(5)=C(6) and C(13)=C(14) show saturation of the 5,6-*cis*- and 13,14-*trans*-double bonds, respectively. 15-OH/15-C=O and 15-OH/15-H also show the conversion of the 15-hydroxyl group into a 15-carbonyl group and deoxidation of the 15-hydroxyl group, respectively.

[b] Difference of the ρ value of X subtracted from that of Y (the ρ values of PGE_2 and 15-deoxy-PGE_2 are 82 and 8%, respectively).[9]

[c] Ratio of $ID_{50}(X)$ to $ID_{50}(Y)$, where $ID_{50}(X)$ and $ID_{50}(Y)$ are the 50% inhibitory doses of PGs, X and Y, for the binding of [^{3}H]PGE_2 in PGE_2 binding site of fetal calf adrenals, respectively.[16a]

[d] Reciprocal of the ratio of BA(X) to BA(Y), where BA(X) and BA(Y) are the relative binding affinities of PGs, X and Y, for rhesus myometrial PGE_1 binding site, respectively.[17]

[e] Ratio of $IC_{50}(X)$ to $IC_{50}(Y)$, where $IC_{50}(X)$ and $IC_{50}(Y)$ are the 50% inhibitory concentrations of PGs, X and Y, for the binding of [^{3}H]PGD_2 in PGD_2 binding site of rat brain synaptic membrances, respectively.[18]

[f] Ratio of $K_d(X)$ to $K_d(Y)$, where $K_d(X)$ and $K_d(Y)$ are the dissociation constants for the binding of PGs, X and Y, to $PGF_{2\alpha}$ binding site of bovine (ovine in parentheses) corpora lutea, respectively.[19]

[g] Ratio of $IC_{50}(X)$ to $IC_{50}(Y)$, where $IC_{50}(X)$ and $IC_{50}(Y)$ are the IC_{50} values of PGs, X and Y, for the binding [^{3}H]$PGF_{2\alpha}$ ([^{3}H]PGE_1 in parentheses) in the primary PG(PPG) binding site of rat vascular smooth muscle cells, respectively.[20]

[h] The ρ value (83%) of $PGF_{2\alpha}$ agrees with that (82%) of PGE_2, but the value (36%) of 15-deoxy-$PGF_{2\alpha}$ is much larger than that (8%) of 15-deoxy-PGE_2 because the former has an additional 9-hydroxy group.[9] This value was not suitable for inclusion in this study.

(1) Saturating the 5,6-*cis*-double bond causes a decrease in the ρ value of 24% because the degree of freedom in the motions of the α-side chain increases. This saturation decreases the binding activities to 1/28 and 1/40 or 1/20 of those of PGD_2 and $PGF_{2\alpha}$ for the corresponding PG binding sites, respectively. Similarly, this saturation decreases the binding activity to 1/16 or 1/13 of that of PGE_2 for the $PGF_{2\alpha}$ binding site, but shows little decrease for other PG binding sites.

(2) Saturating the 13,14-*trans*-double bond causes a 27% decrease in the ρ value because the degree of freedom in the motions of the ω-side chain increases, but the decrease of the ρ value is 17% in the PGE_1 type of compound. This lower value of 17% would be due to an increase in the degree of freedom in the motions of the α-side chain. Saturation of the 13,14-*trans*-double bond reduces the binding activities to 1/4 or 1/2 and 1/36 or 1/14 of those of $PGF_{2\alpha}$ for the $PGF_{2\alpha}$ and PPG binding sites, respectively, and to 1/2 and 1/3 or 1/2 of those of 15-keto-$PGF_{2\alpha}$ for these binding sites, respectively. This saturation also reduces the binding activity to 1/5 of that of PGE_1 for its binding site, but does not decrease that of 15-keto-PGE_1 (**2**).

(3) Saturating both the 5,6-*cis*- and 13,14-*trans*-double bonds causes large decreases of the ρ value of 41% and reduces the binding activity to 1/10 of that of PGE_2 for the PGE_1 binding site.

(4) When the 15-hydroxyl group is converted into a carbonyl group, the ρ value of 14% decreases. This reduces the binding activities to 1/56 and 1/12 of those of PGE_1 and 13,14-dihydro-PGE_1 for the PGE_1 binding site, respectively. Similarly, this conversion decreases the binding activities to 1/180 (1/115 or 1/91) and 1/70 or 1/48 (1/8 or 1/12) of those of $PGF_{2\alpha}$ and 13,14-dihydro-$PGF_{2\alpha}$ for the $PGF_{2\alpha}$ (PPG in parentheses) binding sites, respectively. In the same binding site, these values for 13,14-dihydro-PGE_1 and 13,14-dihydro-$PGF_{2\alpha}$ are larger than those for PGE_1 and $PGF_{2\alpha}$, respectively. This would be due to an increase in the degree of freedom in the motions of the ω-side chain.

(5) When the 15-hydroxyl group is deoxidized, the ρ value of 74% decreases. This effect decreases the binding activity to 1/418 or 1/91 of that of $PGF_{2\alpha}$ for the PPG binding site.

In (4) and (5), the marked decrease of these binding activities is thought to be due to the disappearance of an essential interaction between the 15-hydroxyl group and the PG binding site. Also, although the 5,6-*cis*- and 13,14-*trans*-double bonds may possess some binding activities for the PG binding site, they are disregarded in this study. The configuration of the 15-hydroxyl group is not discussed because it has been reported elsewhere.[9]

According to our hypothesis, described in the Introduction, it is presumed that the larger the degree of freedom is for the motions of the α- and ω-side chains in PGs with decreasing ρ value, the less suitable these compounds are for their binding sites in the receptors. This agreed with our results because the binding activities of PGs decrease as their ρ values decrease. This suggests that, in PGs, the conformation suitable for the binding sites in the receptors, as described in Table 2, is maintained with the intramolecular hydrogen bonds formed between the carboxyl and 15-hydroxyl or 15-carbonyl groups.

PGE_2 has been found to bind to the platelet receptor of prostacyclin (PGI_2),[28] although its molecular structure differs from that of PGI_2 with a bicyclic ring.[29] We reported[9] that PGE_2 includes a small amount (several per cent) of the conformation with the 13-membered ring formed by the intramolecular hydrogen bond (I) between the carboxyl and 11-hydroxyl groups, and this conformation is similar to that observed for carbacyclin,[30] which is a PGI_2 receptor agonist.[31] It has been reported[32,33] that the binding activities of PGEs for the PGI_2 binding sites in various receptors markedly decrease in the order of PGE_1, 5,6-*trans*-PGE_2, and PGE_2. As this order did not agree with our results, we carried out the following examination.

Chain compounds **8**–**10** and 5,6-*trans*-PGE_2 (**11**) form the large-membered ring in which the carboxyl and hydroxyl groups are joined by an intramolecular hydrogen bond (I) similar to that observed for PGs.[9,13,34] In Figure 7, the ρ values were plotted against the size (*S*) of the ring formed by the hydrogen bond (I) in chain compounds, PGEs, and carbacyclin. The ρ value of PGE_2 is much larger than that of **9** which forms the 15-membered ring. However, although the ρ value of carbacyclin, which has the rigid moiety of a 3-methylene-*cis*-bicyclo[3.3.0]octane ring, is larger than that of **8** which forms the 13-membered ring, the ρ value of 15-deoxy-PGE_2 is smaller, in spite of the fact that 15-deoxy-PGE_2 has rigid moieties, a 5,6-double bond and a cyclopentane ring. These findings indicate that, in the formation of the 13-membered ring, steric hindrance around the 5,6-double bond contributes to weakening of the intramolecular hydrogen bond (I). In addition, it is presumed that the percentage of the 13-membered ring conformer in PGE_1, **11**, and PGE_2 decreases as the ρ value increases because these compounds mainly exist in the 15-membered ring conformer and its conformer exists in equilibrium with the 13-membered ring one. The ρ value becomes larger in the order of PGE_1, **11**, and PGE_2. These findings suggest that the percentage of the 13-membered ring conformer becomes smaller in this order. This does not contradict with our hypothesis because the binding activity of these compounds for the PGI_2 binding site decreases as this percentage decreases.

In conclusion, the FTIR method should be useful for elucidating the conformation of PGs in CCl_4 ($d = ca$ 2.2).[10] This conformation should be useful for evaluating conformational analyses using theoretical calculations which are usually carried out under vacuum ($d = 1$). The information obtained is expected to be helpful for

estimating the active conformation of PGs and for designing drugs.

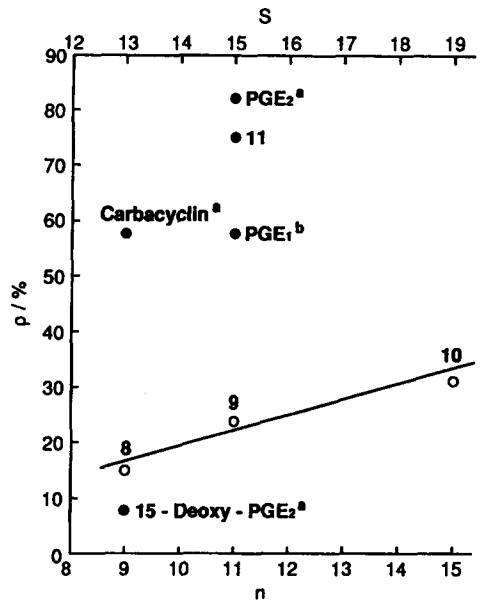


Figure 7. Variation of ρ (○) with chain length for $HO(CH_2)_nCO_2H$ in CCl_4 at concentrations below 4.5×10^{-5} mol dm^{-3}, where *n* is the number of carbon atoms separating functional groups and *S* is the size of a ring formed by the intramolecular hydrogen bond (I). Plots of ρ (●) against *S* in PGEs and carbacyclin. [a]Ref. 9. [b]Estimated ρ value because of very low solubility in CCl_4.[27]

Experimental

Compounds **1–4** and **11** were obtained commercially from Cayman Chemical Co., **6** from Nacalai Tesque, Ltd, **8** from Sigma Chemical Co., and **9** and **10** from Aldrich Chemical Co. Compound **5** was obtained by hydrolysis of **6**. Compound **7** was synthesized at our laboratory.[22] These compounds were dissolved in CCl_4 at a concentration (*c*) below 4.5×10^{-5} mol dm^{-3} (cell length = 5.0 cm) in order to decrease the formation of carboxylic acid dimer as much as possible. The solvent CCl_4 was dried over 4-Å molecular sieves and purified by distillation. All operations on the solution were performed in a dry box filled with N_2 gas. FTIR spectra were recorded on a Nicolet 20SXB FTIR spectrometer at 27 °C. The curve-fitting calculation for peak separation of these spectra was carried out using the Nicolet FOCAS program.

The values of the molar absorption coefficients (ε/mol^{-1} cm^3 cm^{-1}) of the free hydroxyl and the free carbonyl stretching vibration band (free ν_{OH} and free $\nu_{C=O}$ bands, respectively) for the carboxyl group in **1–5** and **7–11** were assumed to be equal to the ε values of the bands at 3533 and 1759 cm^{-1} in lauric acid, respectively.[13] The percentages (*N*) of non-hydrogen-bonded molecules and (σ) of the carboxylic acid dimers for these compounds were estimated using these ε values and the equations shown in Table 1, footnote d,[14,23] respectively. These compounds in CCl_4 at *c* below 4.5×10^{-5} M do not form the intermolecular hydrogen bonds between the functional groups, except for the carboxylic acid dimer.[13,14] Thus, we calculated the percentages [ρ (=100 - *N* - σ)] of their intramolecular hydrogen-bonded molecules from both of the estimated values. These ρ values could be reproduced within ±2% by several measurements.

Acknowledgements

We thank Dr M. Kishi for providing 15-deoxy-PGA_2. The cooperation of Mrs M. Sugita is gratefully acknowledged.

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10. Assuming that a binding site where prostaglandin-type molecules bind is a hole in protein, it is presumed that the dielectric constant (*d*) in the hole would be close to that of protein. The continuous *d* value of protein was assumed to be in the range 2–5 (Gelin, B. R.; Karplus, M. *Biochemistry* **1979**, *18*, 1256) or to be *ca* 3.5 (Rogers, N. K.; Sternberg, M. J. *J. Mol. Biol.* **1984**, *174*, 527) or was theoretically estimated to be *ca* 2.5 (Nakamura, H.; Sakamoto, T.; Wada, A. *Protein Engineer* **1988**, *2*, 177), which is nearly equal to that of CCl_4 ($d = 2.228$). Accordingly, we reported that CCl_4 is suitable as an environment for the binding site.[9,13–15] Another suitable environment has been reported to be that of a non-polar hydrocarbon phase ($d \doteqdot 2$) (Wilkinson, A. J.; Warwick, C. M.; Davies, R. H. *Int. J. Quant. Chem. Quantum Biol. Symp.* **1988**, *15*, 67). The *d* value in the cavity of cyclodextrins known as a mimic of an enzyme was assumed to be 2 (Furuki, T.; Hosokawa, F.; Saburai, M.; Inoue, Y.; Chûjô, R. *J. Am. Chem. Soc.* **1993**, *115*, 2903; Sakurai, M.; Hoshi, H.; Inoue, Y.; Chûjô, R. *Chem. Phys. Lett.* **1989**, *163*, 217).

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26. Since **1–4** form the intramolecular hydrogen bond between the 11-hydroxyl group and the π-electrons on the 9-carbonyl group[24a,b] and between the 11-hydroxyl and carboxyl groups,[9] and **3** and **4** form an additional intramolecular hydrogen bond between the 11-hydroxyl and 15-carbonyl groups, the ρ value of the additional one could not be estimated.

27. The ρ values of PGE_2 and 12-hydroxystearic acid $[CH_3(CH_2)_5CH(OH)(CH_2)_{10}CO_2H]$, which form the 15-membered ring via the intramolecular hydrogen bond (I), were estimated to be 82 and 24%, respectively.[9,15] In these compounds, the ρ value decreases by 14% when the 15- or 12-hydroxyl group is converted into a carbonyl group because the ρ values of **1** and **5** are 68 and 10%, respectively. This finding suggests that the differences between the ρ values of **1–4** are equal to those of the corresponding compounds which have the 15-hydroxyl group. On the basis of this suggestion, the ρ value of PGE_1 was estimated to be 58% from that of 15-keto-PGE_1 because PGE_1 is only slightly soluble in CCl_4.

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34. Since the intramolecular hydrogen bond (I) has been reported for PGs and their related compounds,[9,13] it is not discussed.

(*Received in Japan* 2 *December* 1994; *accepted* 25 *January* 1995)