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Unnatural prostaglandins of biochemical and physiological significance

R. Noyori,† H. Koyano,† M. Mori,† R. Hirata,† Y. Shiga,† T. Kokura,† and M. Suzuki‡

†Department of Chemistry, Nagoya University, Chikusa, Nagoya 464-01, Japan, and ‡Department of Applied Chemistry, Gifu University, Gifu 501-11, Japan

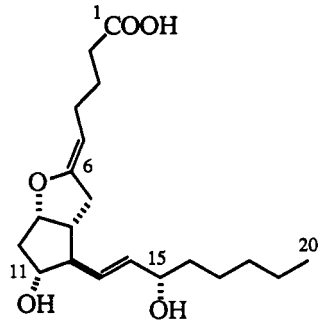
Abstract: Some artificial prostaglandins (PGs) synthesized by the three-component process possess significant biological properties. Isocarbacyclin, a stable analogue of PGI_2, is a promising agent for the treatment of various thrombotic diseases. 19-(3-Azidophenyl)-20-norisocarbacyclin (APNIC) has a sufficiently high affinity to the prostacyclin receptor protein in mastocytoma P-815 cells, exhibiting an IC_{50} value of 3 nM for the replacement of iloprost. Photoaffinity labeling experiments using 15-tritium-labeled APNIC have resulted in the characterization of the PGI_2 receptor protein. Δ^7-PGA_1 methyl ester exhibits unique anti-tumor and anti-viral activities independent of cAMP levels. The dienone PGs are transported reversibly into cultured L1210 leukemia cells and then accumulate in nuclei via covalent interaction, eliciting growth inhibition. This cellular behavior is well correlated with the chemical behavior in the presence of thiols. The dienone undergoes a reversible Michael reaction with various thiols in the homogeneous phase, whereas the reaction with polymer-anchored thiols occurs in an irreversible manner. PGA_1 methyl ester, a less potent enone PG, reacts with thiols at a lower rate, but the resulting adducts are more stable than the dienone adducts.

INTRODUCTION

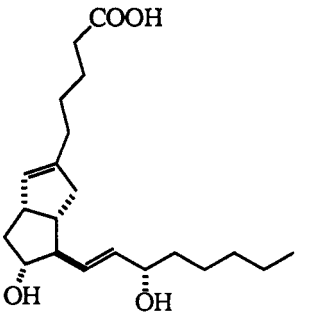
Prostaglandins (PGs) control a wide array of physiological functions in the circulatory, respiratory, and digestive systems and are also crucial for vital defense processes such as inflammation, tissue repair, and immune responses. Recent discoveries include their sleep-inducing effect and anti-neoplastic activity which provides new opportunities for chemotherapy. Thus this family of C_{20} unsaturated polyoxygenated fatty acids has attracted broad attention in chemistry and various biological sciences. Although scientists have concentrated mainly on naturally occurring PGs, certain well-designed artificial analogues exhibit remarkable biological properties. Precise molecular investigations based on organic synthesis open a way for the rational creation of selective therapeutic agents. In this context the three-component synthesis[1] via organometallic chemistry plays a powerful methodological role in exploring a new phase of PG pharmacology.[2]

SYNTHESIS OF ISOCARBACYCLIN

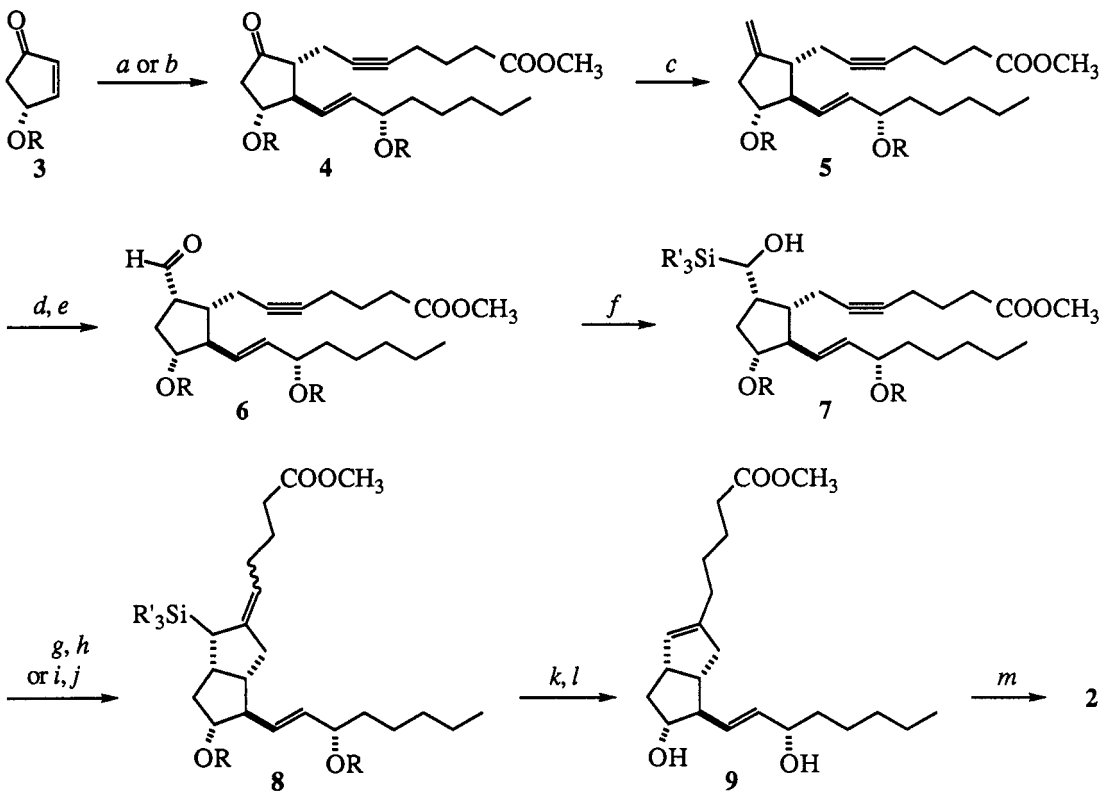
Prostacyclin (PGI_2) (**1**) is a particularly potent vasodilator and inhibitor of platelet aggregation. However, the high sensitivity to hydrolytic destruction of the 2-alkylidenetetrahydrofuran ring has prevented its use as a therapeutic agent. Isocarbacyclin (**2**), a carbocyclic analogue of **1**, which overcomes this stability problem yet maintains sufficient physiological activity, is a promising agent for various thrombotic diseases.[3] In fact clinical trials are now in progress for the treatment of cerebral ischemic diseases and peripheral vascular diseases. Scheme 1 outlines the synthesis of this significant unnatural compound using the three-component process, **3** → **4**.[1,4,5] The requisite chiral building blocks are obtainable by various asymmetric syntheses.[6] The bicyclo[3.3.0]octane skeleton was efficiently constructed by a controlled



1, prostacyclin (PGI_2)



2, isocarbacyclin



R = $Si(CH_3)_2$-*t*-C_4H_9
SiR'_3 = $Si(CH_3)_2C_6H_5$

a: (1) (1*E*,3*S*)-LiCH=CHCH[$OSi(CH_3)_2$-*t*-C_4H_9]-*n*-C_5H_{11}, $Zn(CH_3)_2$, THF, –78 °C; (2) $ICH_2C{\equiv}C(CH_2)_3COOCH_3$, HMPA, –40 °C; 71%. *b*: (1) (1*E*,3*S*)-LiCH=CHCH[$OSi(CH_3)_2$-*t*-C_4H_9]-*n*-C_5H_{11}, CuI, (*n*-$C_4H_9)_3P$, THF–ether, –78 °C; (2) $(C_6H_5)_3SnCl$, HMPA, –78 °C; (3) $ICH_2C{\equiv}C(CH_2)_3COOCH_3$, HMPA, –30 °C; 82%. *c*: Zn–CH_2Br_2–$TiCl_4$, CH_2Cl_2, 0 °C, 97%. *d*: (1) 9-BBN, CH_3COOCH_3, THF, 0 °C; (2) NaOH, H_2O_2, 0 °C; 84%. *e*: pyridinium dichromate, CH_2Cl_2, 85%. *f*: $LiCu[Si(CH_3)_2C_6H_5]_2(CN)$, THF, –78 °C, 85%. *g*: *m*-$CF_3C_6H_4COCl$, 4-(dimethylamino)pyridine, CH_3CN, 90%. *h*: *hν*, *N*-methylcarbazole, $Mg(ClO_4)_2$, THF–H_2O, 75%. *i*: (1) LiN(*i*-$C_3H_7)_2$, THF, –78 °C; (2) CS_2, HMPA, –78 °C; (3) CH_3I, 0 °C; 68%. *j*: (*n*-$C_4H_9)_3SnH$, cat. (*t*-$C_4H_9O)_2$, benzene, 65 °C, 86%. *k*: aq $HClO_4$, CH_3OH–ether, 98%. *l*: CF_3COOH, CH_2Cl_2, –78 to –20 °C, 96%. *m*: aq NaOH–CH_3OH, 100%.

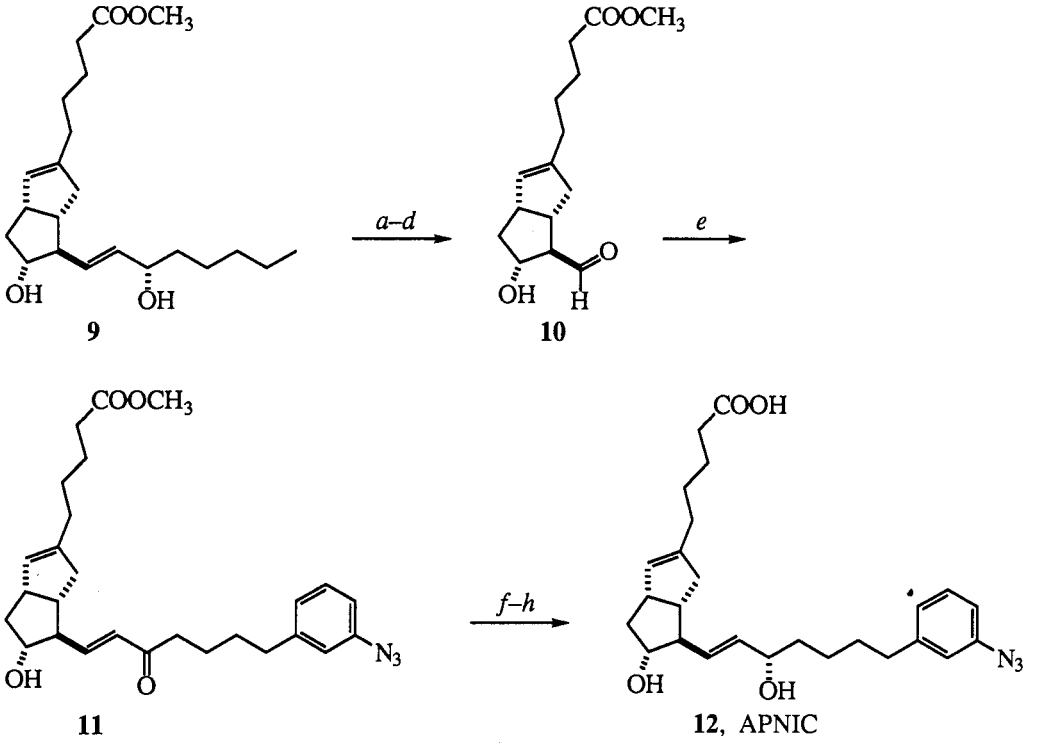
Scheme 1. Synthesis of isocarbacyclin

radical cyclization, **7** → **8**, and the protodesilylation of the allylsilane **8** allowed the selective placement of the C=C bond at the desired position.[7]

DESIGN AND SYNTHESIS OF A PHOTOAFFINITY PROBE FOR A PROSTACYCLIN RECEPTOR

The diverse biological activities of PGs emerge via a series of signal transductions initiated by the binding of PGs to the corresponding specific receptor proteins in cell membranes. Precise molecular level investigations required to create therapeutic agents having enhanced metabolic stability and higher tissue selectivity. PGI_2 activates adenylate cyclase via Gs proteins to promote the cAMP production. Unfortunately, little is known about the structures of the PGI_2 receptor proteins and their functions because of their low concentrations in cell membranes and the lack of suitable agonists or antagonists to purify a receptor protein in addition to the instability of PGI_2. We have developed for the first time a stable, very efficient photoaffinity probe for a PGI_2 receptor protein through structural modification of readily accessible isocarbacyclin methyl ester (**9**).[8]

Assuming that the PGI_2 receptor protein recognizes the terminal carboxyl group, sp^2-hybridized C(6) atom, chiral cyclopentane ring, and the 11- and 15-hydroxyl groups of **1**, we planned to incorporate an azidophenyl group into the terminus of the ω side chain of the stable analogue **2**. The initial target was the standard structure of the ω side chain comprising the zigzag-oriented eight carbon atoms including three in the aromatic ring. We further investigated the influence of the chain length and the position of azido function on the aromatic ring to the affinity for the PGI_2 receptor protein. These compounds can be



a: $Ti(O\text{-}i\text{-}C_3H_7)_4$, L-(+)-diisopropyl tartrate, $(CH_3)_3COOH$, CH_2Cl_2, –20 °C, 93%. *b*: $(CH_3CO)_2O$, 4-(dimethylamino)pyridine, CH_2Cl_2, 96%. *c*: (1) CH_3COOH–H_2O, 100 °C; (2) K_2CO_3–H_2O; 92%. *d*: $NaIO_4$, THF–ether–H_2O, 96%. *e*: NaH, DME–toluene, 92%. *f*: (1) $NaBH_4$–$CeCl_3$, CH_3OH, 0 °C; (2) chromatographic separation of C(15) diastereomers; 38%. *g*: LiOH, H_2O–CH_3OH, 98%.

Scheme 2. Synthesis of APNIC

prepared by selective oxidative cleavage of the ω side chain of **9**, giving the aldehyde **10**, and reconstruction of the chain structure using appropriate azide-containing Horner–Emmons reagents. As a consequence, we found that 19-(3-azidophenyl)-20-norisocarbacyclin (**12**), abbreviated as APNIC, has a sufficiently high specific affinity to the PGI_2 receptor protein in mastocytoma P-815 cells, exhibiting an IC_{50} value of 3 nM for the replacement of iloprost bound to the receptor protein.[9] Scheme 2 illustrates the synthetic sequence. The C(15) absolute configuration was determined by chemical correlation to optically pure glycerol acetonide. Interestingly, APNIC having a "ten-carbon" ω side chain showed higher affinity than other analogues with a standard "eight-carbon" side chain. Furthermore, this compound appeared to act as an agonist for the PGI_2 receptor as judged by dose-dependent stimulation of adenylate cyclase in the presence of guanosine triphosphate.[9]

Investigation using the isotope-labeled compound, [^{3}H]APNIC, obtained via reduction of the C(15) ketonic precursor **11** by a [^{3}H]$NaBH_4$–$CeCl_3$ mixture, revealed that (1) specific binding of **12** for the PGI_2 receptor is ca. 70% for total binding; (2) **12** binds selectively to the PGI_2 receptor in mastocytoma membrane but much more weakly to the coexistent PGE_2 receptor; (3) it interacts with a single binding site with a dissociation constant, K_d, of 4.7 nM and a maximum binding, B_{max}, of 0.58 pmol/mg protein (Scatchard analysis); (4) **12** binds to the PGI_2 receptor coupled with GTP binding proteins.[9]

Photoaffinity labeling of the receptor protein was conducted by incubation of the radioligand, [^{3}H]APNIC, with the plasma membrane of mouse mastocytoma P-815 cells followed by irradiation with a UV lamp (254 nm).[9] Filter assays indicated that 80% of the specific binding of **12** to mastocytoma membrane had been photolabeled. Subsequent sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis indicated a major peak at around 43 kDa visualized by fluorography. The incorporation of radioactivity to the 43 kDa protein band had been completely inhibited by iloprost, unlabeled APNIC (**12**), and isocarbacyclin (**2**) and to a slightly lesser extent by PGE_1. PGE_2, PGD_2, and $PGF_{2\alpha}$ did not affect the photoincorporation of this protein. Photolabeling was also attenuated by GTPγS. These results support the idea that this protein corresponds to the PGI_2 receptor. The strong platelet-aggregation inhibition by PGI_2 prompted us to extend this technique to porcine and human platelet membranes. Consequently, the specific photolabeling of a 45 kDa (Fig. 1)[9] and 52 kDa[10] proteins was realized. These PGI_2 receptor proteins are thought to be glycoproteins based on their broad bands. Thus the PGI_2 receptor was unambiguously identified for the first time by using a newly devised irreversible specific photoaffinity probe.

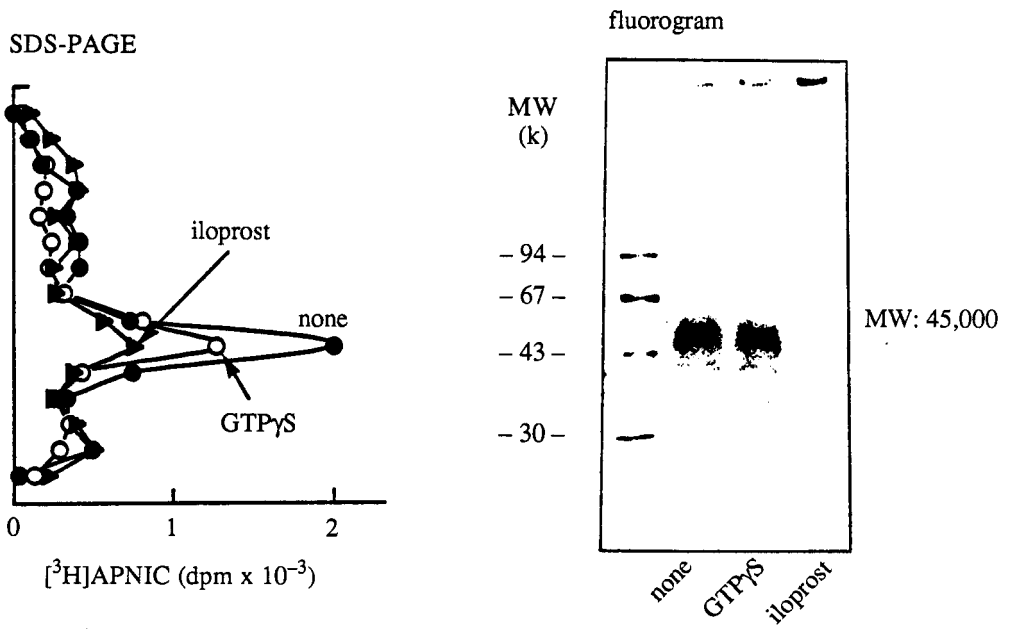
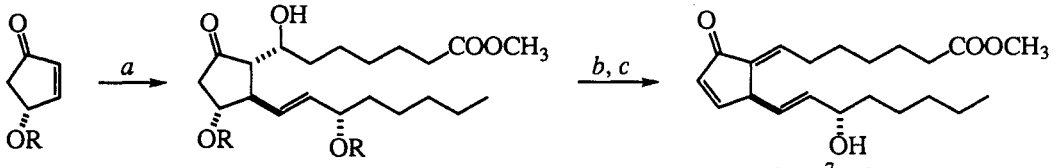


Fig. 1. SDS-PAGE and fluorogram of labeled proteins for porcine platelet

CHEMICAL IMPLICATIONS FOR ANTI-TUMOR AND ANTI-VIRAL PROSTAGLANDINS

Some cyclopentenone PGs show potent anti-tumor and anti-viral effects without affecting cAMP levels.[11] For instance, Δ^7-PGA_1 methyl ester (**14**) and Δ^{12}-PGJ_2 (**15**), unconventional dienone PGs, commonly prohibit growth of L1210 leukemia cell with IC_{50} values of 0.3 and 0.7 μg/mL, respectively, and also inhibit primary transcription of HSV-2 with ID_{50} of 0.35 and 1.8 μg/mL. An extensive study of the structure/activity relationship indicated that the presence of the cross-conjugated dienone system is essential for the exertion of the potent growth-inhibiting activity; simple 2-cyclopentenone and 2-alkylidenecyclopentanone derivatives are active but with lower potency.[12] Such PG activities at non-cytotoxic doses may provide new therapeutic strategies. The cross-conjugated dienone **14** which is readily accessible by the three-component synthesis using a C_7 aldehyde as the α side-chain unit, as shown in Scheme 3 (aldol route),[5,13] is now under preclinical investigation for the treatment of chemotherapeutically resistant ovarian cancer. This dienone slowly arrests the cell cycle at the G_1 phase after doing.



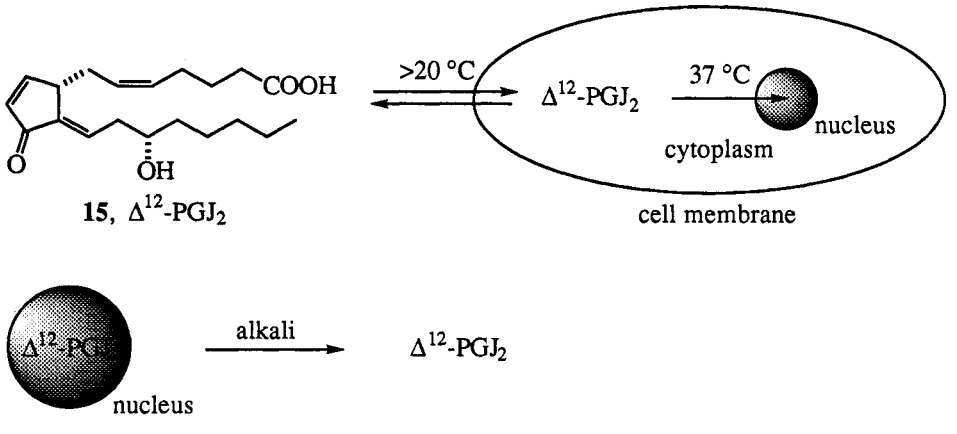
3 **13** **14**, Δ^7-PGA_1 methyl ester

R = $Si(CH_3)_2$-*t*-C_4H_9

a: (1) (1*E*,3*S*)-LiCH=CHCH[$OSi(CH_3)_2$-*t*-C_4H_9]-*n*-C_5H_{11}, $Zn(CH_3)_2$, THF, –78 °C; (2) $HCO(CH_2)_5COOCH_3$, –78 to –30 °C; 92% (a 10:1 mixture of 7*S* and 7*R* diastereomers). *b*: CH_3SO_2Cl, 4-(dimethylamino)pyridine, CH_2Cl_2, 80%. *c*: 2:1:1 CH_3COOH–THF–H_2O, 60 °C, 72%.

Scheme 3. Synthesis of Δ^7-PGA_1 methyl ester

The detailed study of the behavior of the dienone PGs using cultured L1210 cells revealed the presence of a unique mechanism for the cellular uptake and nuclear accumulation outlined in Scheme 4.[14] The pharmaco-kinetic investigation of **15** indicated that: (1) the PG undergoes reversible carrier-mediated incorporation to the cells, establishing a rapid influx–efflux system at >20 °C; (2) a considerable medium/cell concentration gradient is present, favoring cellular uptake by a factor of up to 20; (3) the intracellular PG is incorporated into nuclei at 37 °C without metabolization; (4) the PG is eventually bound

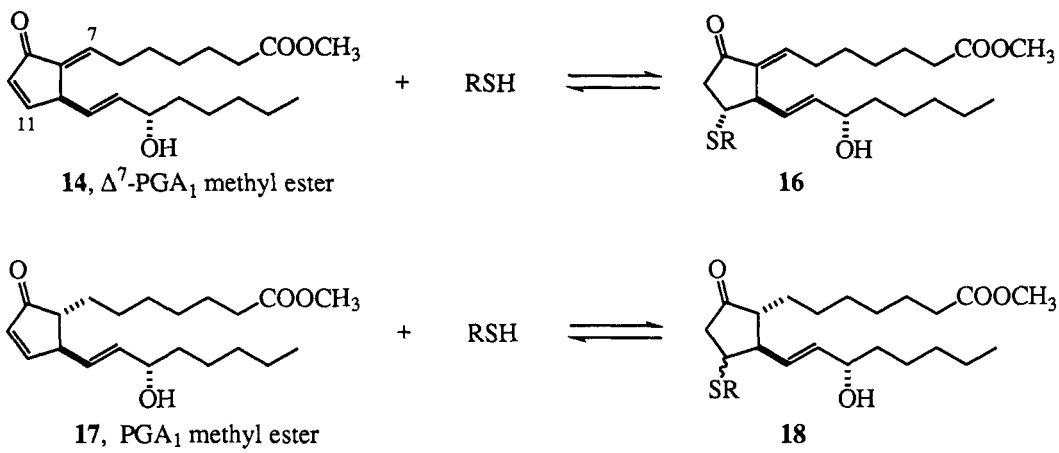


15, Δ^{12}-PGJ_2

Scheme 4. Behavior of Δ^{12}-PGJ_2 at cell level

to nuclear proteins through a covalent bond. The final process is irreversible under physiological conditions, while alkali treatment effects the dissociation to the free PG. Structurally related **14** and **15** show the same characteristics. PGA_2, a simple enone analogue, is transported into the cells and nuclei in a similar manner but is bound to the nuclei to a lesser extent. We have been interested in the chemical interpretation of such biological properties of the unsaturated PGs, particularly their cellular behavior, nature of the target molecules, and the origin of the difference in potency between the dienone and enone compounds.[2]

We first examined the reaction of the dienone **14** with various nucleophiles under conditions mimicking the physiological system (phosphate buffer, pH 7.4) and found that only thiols such as methanethiol, butanethiol, mercaptoethanol, mercaptoacetic acid, cysteine, and glutathione, react with **14** (Scheme 5). The Michael addition took place selectively at the C(11) position, leading to products of type **16**. The MO calculation using (*E*)-4-methyl-5-ethylidene-2-cyclopentenone as a model suggests that this regioselectivity is due to a larger LUMO coefficient and less net atomic charge for the carbon corresponding to the PG C(11) position with respect to C(7). With excess thiols, the 7,11-bis-thiol adducts were formed. The dienone was inert to non-thiol biomolecules such as nucleotides, carbohydrates, and amino acids. PGA_1 methyl ester (**17**), an enone, reacted with thiols similarly to form the adducts **18**. A kinetic study indicated that the conjugate addition of mercaptoethanol to the dienone **14** at 25 °C proceeded six-times faster than to the enone **17**. The reduction potentials (E_p), –1.64 and –1.96 V for **14** and **17**, respectively, reflect well on their reactivities. The addition is reversible and, notably, the dienone–thiol adduct is less stable than the enone–thiol adduct as judged by the dissociation constants (K_d), 3.3 mM and 0.11 mM for **16** and **18**, respectively. The glutathione adducts behave similarly, K_d(**16**) = 2.9 mM vs K_d(**18**) = 0.16 mM. Thus the dienone **14** reacts with thiols faster than the enone **17** but, under thermodynamic conditions, the former exists in its free state to a greater extent than does the latter.

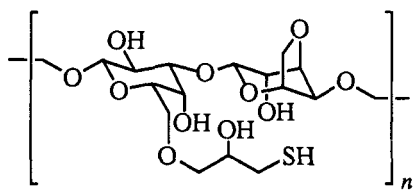


14, Δ^7-PGA_1 methyl ester **16**

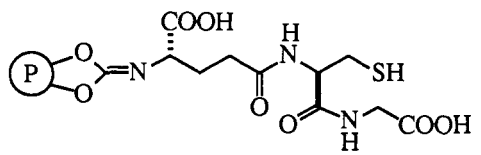
17, PGA_1 methyl ester **18**

Scheme 5. Reaction of anti-tumor PGs and thiols in CH_3OH (pH 7.4 buffer)

Polymer-anchored thiols may mimic biological thiols in nuclei. The dienone **14** dissolved in methanol (pH 7.4 buffer, 25 °C) reacted with Sepharose-bound mercaptoethanol or glutathione (**19** and **20**, respectively)



19, Thiopropyl-Sepharose 6B (reduced form)



20, Activated Thiol-Sepharose 4B (reduced form)

faster than the enone **17** by a factor of 3. The formation of the covalent bond was confirmed by the FT-IR spectroscopy using a diffuse reflectance measurement technique. Notably, unlike in the homogeneous phase, the resulting polymer-anchored Michael adducts of type **16** and **18** are stable at pH 7.4 and dissociate into the free thiols and PGs only under basic conditions at pH >9.5.

The cellular behavior of the dienone and enone PGs are qualitatively similar to their purely chemical kinetic and thermodynamic properties. When 0.01 mM of PG **15** or PGA_2 is incubated into L1210 cells, a maximum 0.2 mM of intracellular PG concentration is attained.[14a] The intracellular **15** reacts readily with small, soluble thiols in a reversible manner. The present chemical study using **14** as a model suggests that, since the cytoplasm contains ca. 2.8 mM of glutathione,[14a,15] 48% of the PG **15** exists as the glutathione adduct and the remaining 52% in a free state. This reversible Michael reaction allows influxing–effluxing of the PG through cellular membranes together with transport to the nuclei. The covalently bound thiol adduct in nuclei in turn remains stable because of the decreased molecular motion. The accumulated PGs can be dissociated from the polymeric biomatrices only by alkali treatment. This is also the case with simple enone PGs. However, as judged from the chemical reaction of the analogue **17**, 94% of the intracellular PGA_2 is bound to the thiol and only 6% of the enone is free. Thus obviously the dienone PGs can reach nuclei and react more efficiently with nuclear thiols, exhibiting higher potency. The purely chemical system is different from the biological environment but nevertheless would help the understanding of the cellular behavior of the anti-cancer PGs.

Acknowledgment

Identification of the prostacyclin receptor protein was done in collaboration with Professor A. Ichikawa and Drs. M. Negishi and H. Hashimoto at the Department of Physiological Chemistry of Kyoto University and Dr. S. Ito of Osaka Bioscience Institute. We are grateful to Dr. M. Fukushima, Aichi Cancer Center, and Professor S. Narumiya, Kyoto University, for their valuable suggestions.

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