Azaprostanoic Acid Derivatives. Inhibitors of Arachidonic Acid Induced Platelet Aggregation[1]

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Received November 6, 1978

A series of 13-azaprostanoic acids (4a–h) and a 15-azaprostanoic acid (11a) have been prepared. Synthesis of the 15-aza derivative is based on a novel transformation of a ketone to an N-substituted ethylenamine using a formylmethylimino phosphate derivative. Several of the azaprostanoic acid derivatives were found to be potent inhibitors of platelet aggregation induced by arachidonic acid, whereas no effect was observed on ADP-induced primary aggregation, indicating blockade of the platelet arachidonic acid cascade. The compounds do not inhibit bovine cyclooxygenase activity and are postulated as acting beyond the synthesis of the prostaglandin endoperoxides. The inhibitory effect of the 13-aza series is highly sensitive to both stereochemistry and length of the amino side chain. Any deviation from the natural prostaglandin skeletal arrangement results in decreased biological activity.

Exciting new developments over the last few years pointing to the pivotal role of prostaglandins (PG) in the irreversible, secondary phase of platelet aggregation have provided a rationale for the design of drugs potentially useful for the treatment of thrombotic conditions such as myocardial infarction, stroke, and pulmonary embolism. Platelet aggregation is involved in both hemostasis and thrombosis, and the initial events which lead to the formation of a platelet plug or thrombus appear to be similar in both instances.[2]

The aggregation of human platelets induced by adenosine diphosphate, adrenaline, or thrombin in citrated plasma occurs in two phases.[3,4] The second phase is associated with the secretion of stored amines and nucleotides which are themselves aggregating agents.[5] Compounds which inhibit prostaglandin synthesis[6,7] also inhibit the second phase of aggregation and secretion,[8,9] suggesting that prostaglandin synthesis is necessary for this second irreversible phase of aggregation.

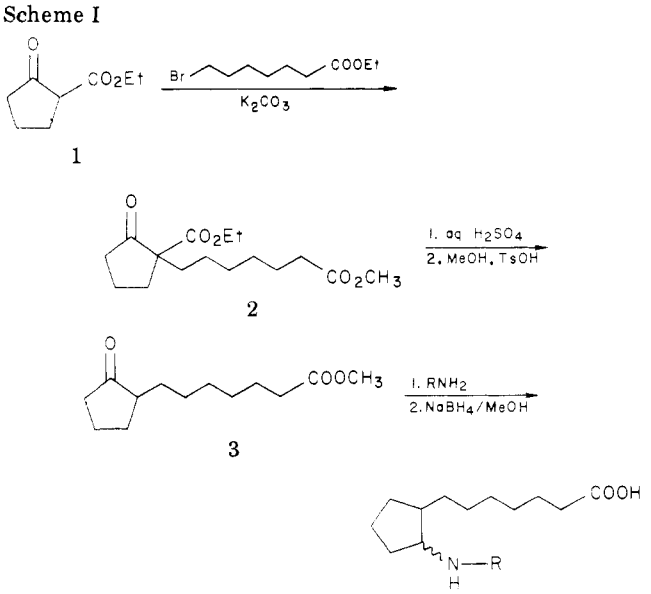
Recently, Samuelsson and co-workers established that aggregation of human platelets induced by collagen or arachidonic acid is mediated through PG endoperoxides (PGG_2 or PGH_2).[10,11] Further study on the metabolism of endoperoxides led to the discovery of a potent inducer of irreversible platelet aggregation, i.e., TXA_2 and its inactive metabolite TXB_2.[12,13] The structure of TXA_2 was postulated by Samuelsson as a bicyclic oxatane ring derivative formed from PG endoperoxides by enzymatic isomerization.[14]

Since the enzymatic conversion of arachidonate to the endoperoxides is the common initial step in the biosynthesis of TXA_2 as well as the potent platelet inhibitor prostacyclin, inhibition of the initial cyclooxygenase reaction would not presumably be as effective in reducing in vivo platelet reactivity as specific inhibition of TXA_2 synthesis or its corresponding receptor.

Several compounds, including the antiinflammatory agent benzydamine,[15] imidazole,[16], 2-isopropyl-3-nicotinylindole,[17] and 9,11-azoprosta-5,13-dienoic acid (Azo I),[18] are known to inhibit PGH_2 conversion to TXA_2 by inhibiting TXA_2 synthetase. In this connection, Azo I has been shown by Fitzpatrick and Gorman to be the most potent and specific of the inhibitors tested.[19] To date, however, no prostaglandin-like compounds are known to act beyond the synthesis of TXA_2.[20]

The present work describes the synthesis and structural assignment of a series of simple azaprostanoic acid derivatives (**4a–h**, Scheme I) which are potent inhibitors of platelet aggregation. Structure–activity data for the series indicates a close correlation with that of the natural prostaglandin skeletal arrangement. Data are also presented indicating that the site of action for these compounds is in the latter stages of the arachidonic acid cascade beyond cyclooxygenase production of the endoperoxides.

Scheme I



4a, R = *trans*-$(CH_2)_6CH_3$
b, R = *cis*-$(CH_2)_6CH_3$
c, R = *trans*-$(CH_2)_5CH_3$
d, R = *cis*-$(CH_2)_5CH_3$
e, R = *trans*-$(CH_2)_5CH_3$
f, R = *cis*-$(CH_2)_5CH_3$
g, R = *trans*-$CH_2C_6H_5$
h, R = *cis*-$CH_2C_6H_5$

Chemistry. Although one can conceive of a number of synthetic approaches to 13-azaprostanoic acids, a most direct and versatile approach would be via the known keto ester **3** (Scheme I). Thus, condensation of this keto ester with a variety of amines, reduction of the Schiff base so formed, and hydrolysis allow considerable freedom in elaborating the lower side chain of the prostaglandin-like structure from a common intermediate. Clearly, the success of this approach requires separation of the anticipated cis and trans isomers produced by the reduction and assignment of stereochemistry.

The key intermediate **3** used in the synthesis of both the 13- and 15-azaprostanoic acids was prepared by the me-

0022-2623/79/1822-0824$01.00/0

thod of Novak and Szantay,[21] which in our hands produced better yields than the earlier procedure reported by Bagli and Bogri.[22] The former approach uses a base-catalyzed alkylation of 2-carbethoxycyclopentanone (1) with methyl 1-bromoheptanoate to give the diester **2** in high isolated yield. The hydrolysis and decarboxylation of the diester **2** was carried out as with the Bagli procedure, but purification of the intermediate acid was found to be unnecessary. Thus, the impure reaction mixture from the hydrolysis and decarboxylation step was esterified directly. Distillation of the esterified reaction mixture gave the keto ester **3** in good overall yield.

With the keto ester **3** in hand, a series of amino esters were prepared. In all cases, we were able to obtain good yields of the ketimines, as judged by GLC analysis, by adding an excess of the amine to a solution of **3** in methanol containing molecular sieves to scavenge the water formed from the condensation. The ketimines were not isolated but directly reduced with sodium borohydride to an approximately equal mixture of *cis*- and *trans*-amino esters as judged by TLC.

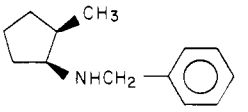
For example, benzylamine was condensed with **3** as described above to give a mixture of amines as expected. A portion of the product was separated by preparative TLC. In addition to the excess starting amine, two distinct bands were separated which gave positive tests when treated with nitroprusside–acetaldehyde spray reagent specific for secondary amines. Both compounds so isolated gave identical mass spectral fragmentation patterns and on the basis of this and the ^{1}H NMR data discussed below were judged to be *cis*-2-(*N*-benzylamino)-1-(6-carbomethoxyhexyl)cyclopentane (**4h**) and the corresponding trans isomer **4g**.

Large-scale separation of **4h** and **4g** was accomplished by column chromatography. The products obtained as oils gave the expected ^{1}H NMR spectra. The only difference observed in the ^{1}H NMR spectra for these isomers was a multiplet (1 H) centered at 3.0 ppm for the less polar isomer, which appeared upfield at 2.6 ppm for the more polar isomer. The chemical shift of this proton indicated that it was the ring proton adjacent to the nitrogen.

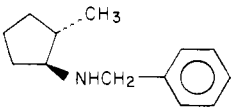
The chemical shifts of vicinal protons, as well as the coupling constants observed for vicinal protons, can be a useful aid in the determination of cis–trans relationships in cyclic ring systems. This situation is particularly well documented in six-membered rings. However, the uncertainty due to the conformational flexibility of most other ring systems, especially four- and five-membered rings, makes stereochemical assignments more difficult. Indeed, in the present situation, involving a five-membered ring and complex second order spin system, application of the usual Karplus relationship would be tenuous at best.

The literature contains ample precedence for the chemical-shift differences of vicinal protons in 1,2-disubstituted five-membered rings.[23-28] In all cases, the resonance signal of the trans protons lies upfield from the shift observed for the corresponding cis protons. The upfield shift of the trans proton may be explained by the anisotropy of the adjacent C–C bond,[29] although this is referred to only in the recent work of Rei.[28] In general, the principal magnetic susceptibility axis of a carbon–carbon single bond lies perpendicular to the bond. Inspection of molecular models indicates that the proton vicinal to the alkyl substituent in trans 1,2-disubstituted cyclopentanes is shielded to a greater extent than the proton in the corresponding cis isomer and thus would be expected to be shifted further upfield relative to the corresponding cis configuration.

In order to definitively establish that a similar relationship holds for cyclopentylamines, we prepared the model compounds **5a** and **5b** by the same procedure used



5a



5b

to prepare the 13-azaprostanoic acids. The stereochemical assignment was then made from an alternate stereospecific synthesis of the trans isomer. Thus, condensation of benzylamine and 2-methylcyclopentanone gave, after reduction and separation, an equimolar amount of **5a** and **5b**. As previously observed, the less polar isomer had a multiplet integrating for one proton centered at 3.0 ppm, whereas for the more polar isomer this multiplet was shifted upfield to 2.5 ppm. The more polar isomer was assigned the trans configuration **5b** based on the previous arguments. The stereochemical assignment for **5b** was confirmed by the synthesis of **5b** by an alternate route. Hydroboration–amination of 1-methylcyclopentane yields *trans*-2-methylcyclopentylamine, which is readily converted to the corresponding benzamide.[30] The benzamide was reduced with Red-Al® to give the *trans*-amine **5b** which was identical in all respects with that prepared from the ketone.

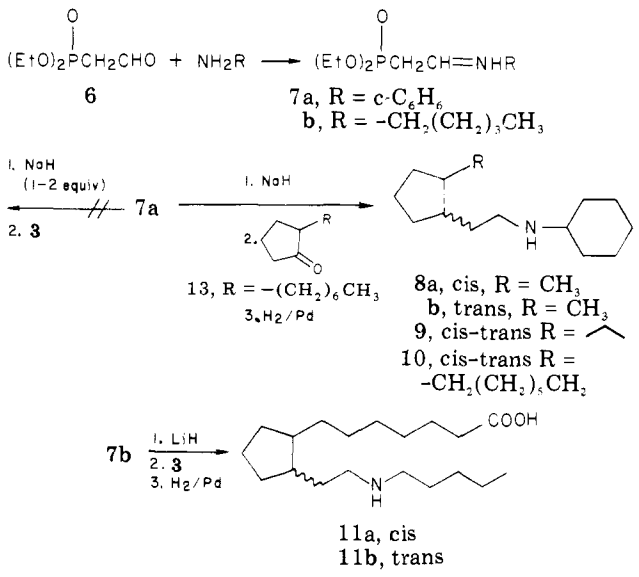
Although the ^{1}H NMR spectra of the azaprostanoic acids were quite uninformative, the mass spectral fragmentation of the 13-azaprostanoic acid derivatives followed predictable routes. The base peak for derivatives **4a–f** was in all cases *m/e* 226, corresponding to the normal α cleavage of amines. A second distinguishing feature of the fragmentation, common to all of the derivatives, was the loss of a fragment corresponding to $C_9H_{17}O_2$ derived from C_8–C_{12} bond cleavage, hydrogen abstraction from C_{11}, and subsequent bond cleavage of C_{11}–C_{10}. Similar fragmentation was observed for the methyl esters, leading to the loss of $C_{10}H_{19}O_2$, and the model compounds **5a** and **5b** losing C_3H_7. This fragmentation is analogous to that previously observed for cyclopentanols.[31]

Our approach to the synthesis of the 15-azaprostanoic acids centered around the availability of the keto ester **3** used in the 13-aza series. The literature does not describe any method for the direct conversion of a ketone to the corresponding N-substituted ethylamine desired in the present work. However, Nagata and Hayase have developed a general procedure for the formylolefination of carbonyl compounds, an intermediate of which seemed applicable to such a direct ethylenamine functionalization.[32] Their procedure employed the addition of a cyclohexyliminovinylphosphonate to carbonyl compounds to give α,β-unsaturated aldimines, which were then hydrolyzed to the corresponding formylolefins. These workers were able to convert even relatively hindered ketones (e.g., 17-keto steroids) to the corresponding formylolefins in good yields, presumably because of the enhanced nucleophilicity of the phosphonate carbanions when compared to that of the phosphonium ylides.

Clearly, if one could vary the *N*-alkyl substituent of the phosphonate while effectively maintaining the Wittig–Horner type of addition, then reduction of the intermediate aldimine should give the desired direct ethylenamine functionalization.

The synthesis of the known[32] cyclohexyliminovinylphosphonate **7a** from formylmethane phosphonate **6** and cyclohexylamine lends itself to the preparation of a variety of alkyl iminophosphonate derivatives (Scheme II). Although bulky amine substituents (e.g., cyclohexylamine)

Scheme II



were previously employed to decrease any tendency of the phosphonate carbanion toward self-addition, it was anticipated that other N-substituents would not drastically alter the course of the reaction.

To test the feasibility of this transformation on the present compounds, the cyclohexylphosphonate **7a** was prepared, converted to the carbanion with sodium hydride, and treated with the keto ester **3**. With either an equimolar or twofold excess of the phosphonate, GLC and TLC analysis of the reaction mixture repeatedly indicated that only starting materials were present. The fact that 17-keto steroids reacted smoothly under these conditions seemed to indicate that the problem was not of a steric nature.[32]

To test the accessibility of the phosphonate carbanion to sterically hindered ketones related to **3**, 2-methylcyclopentanone was treated with an equimolar amount of the sodium salt of **7a**. This yielded an oil, which was catalytically reduced over palladium to an isomeric mixture of the expected amines **8a** and **8b**, which were separated by preparative GLC. In addition, 2-allylcyclopentanone[33] and 2-*n*-heptylcyclopentanone (**13**) readily reacted with the phosphonate carbanion to give, after reduction, the expected amines **9** and **10**, respectively. The fact that each of these reacted smoothly with the carbanion of **7a** without alteration of reaction conditions indicated that steric factors were probably not involved in the inability of **3** to react with the phosphonate carbanion. This implied that the ester function of **3** must, by some mechanism, alter the course of the reaction.

However, a change from the sodium to the lithium salt of **7a** and treatment of the phosphonate carbanion so formed with **3** clearly gives the α,β-unsaturated aldimine in good yield (data not shown). It is not at all clear why this difference between metalation with lithium vs. sodium exists when the ester is present.

Using this modified procedure, an isomeric mixture of 15-azaprostanoic acids, **11a** and **11b**, were prepared. Keto ester **3** was treated with the lithium salt of the pentyl phosphonate derivative **7b**. The phosphonate **7b** was readily prepared by the same procedure used for the preparation of 7a. Apparently, use of the primary vs. secondary alkyl substituent on the phosphonate nitrogen did not lead to any significant self-addition of the phosphonate. The amino esters were hydrolyzed in dilute base and brought to their isoelectric point as with the 13-aza series, and the *cis*- and *trans*-15-azaprostanoic acids

Table I. ^{13}C Chemical Shifts 1-Methyl-2-[2-(*N*-cyclohexylamino)ethyl]cyclopentane (8a)[a]

carbon	isomer: cis (8a)	isomer: trans (8b)
$-CH_3$	13.8	19.1
C_1	35.2	40.5
C_2	40.3	45.6

[a] Chemical shifts in $CHCl_3$ are in parts per million relative to tetramethylsilane.

separated chromatographically in a ratio of approximately 95:5, respectively. The two isomers of 15-azaprostanoic acid gave identical ^1H NMR and mass spectra and were clearly chromatographically different; however, the low yield of the minor isomer did not allow full characterization. It was anticipated that the major isomer obtained from the catalytic reduction of the α,β-unsaturated aldimine would have the cis configuration, with the approach of the catalyst occurring from the least-hindered face of the double bonds.[34] Direct experimental evidence was sought for this assignment in the NMR of the more readily available model compounds **8a** and **8b** prepared in an analogous fashion.

Since the nitrogen in the 15-aza series is now two carbons removed from the ring relative to the 13-aza analogues, the C_{12}-H no longer has the chemical shift identity it had in the 13-aza series. Thus, the anisotropy arguments applied to the previous series are not applicable to the 15-azaprostanoic acids.

Roberts and co-workers have studied the ^{13}C NMR spectra of a number of substituted cyclopentanes and have found ^{13}C-steric shifts potentially useful in the assignment of configuration in five-membered rings.[35] In simple substituted 1,2-cyclopentanes, the carbons directly attached to the ring show an upfield shift of about 5 ppm for the cis isomer when compared to the trans, presumably due to steric interactions of the two substituents. Similarly, the *ring* carbons to which the substituents are attached also show an approximately 5-ppm upfield shift in the cis configuration.

The assignment of the C_1, C_2, and methyl carbons in the model compounds 8a and **8b** follows directly from single-frequency off-resonance decoupled spectra and from model compounds. As may be seen in Table I, these three C resonances are all shifted upfield by 5.3 ppm when the major isomer (**8a**) is compared to the minor isomer (**8b**). Thus, catalytic reduction of the precursor aldimine apparently occurs mainly from the least-hindered face of the double bonds, giving the cis configuration as the major isomer (75%) in the model compounds. Furthermore, this suggests that the major isomer formed from identical reduction conditions leading to the 15-azaprostanoic acids is also the cis isomer **11a**.

Pharmacology. The effect of various azaprostanoic acids on arachidonic acid induced platelet aggregation is shown in Table II. The relative inhibitory activities of the azaprostanoic acids were determined by varying the concentration of arachidonic acid while keeping the inhibitor concentration constant. Thus, at the highest concentration of arachidonate only the *trans*-13-azaprostanoic acid derivative shows any inhibitory activity, while at the lowest concentration the compounds show varying degrees of activity with the benzyl derivative **4g** being inactive.

On the other hand, none of the compounds examined were shown to inhibit ADP-induced primary platelet aggregation. Citrated human platelet-rich plasma (PRP)

Table II. Inhibition of Arachidonic Acid Induced Platelet Aggregation (Human PRP) by Azaprostanoic Acids (10^{-5} M)

no.	stereochem	R	% inhibn,[a] conc arachidonate 500 μM	400 μM	300 μM
4a	trans	$-(CH_2)_6CH_3$	19	66	100
4b	cis	$-(CH_2)_6CH_3$	0	11	43
4c	trans	$-(CH_2)_7CH_3$	0	7	71
4d	cis	$-(CH_2)_7CH_3$	0	11	50
4e	trans	$-(CH_2)_5CH_3$	0	0	14
4f	cis	$-(CH_2)_5CH_3$			
4g	trans	$-CH_2C_6H_5$	0	0	0
4h	cis	$-CH_2C_6H_5$			
11	cis		0	16	91

[a] Values reported were determined on a single blood sample and are representative of several determinations. Absolute inhibition was found to vary from one blood sample to another but relative inhibition by the azaprostanoates remained the same.

was incubated with aspirin (1 mg/mL) for 15 min. Treatment (2 min) of this plasma with 100 μM *cis*- or *trans*-13-azaprostanoic acid, *trans*-13-aza-*N*-benzyl derivatives **4g** and **4h**, or *cis*-15-azaprostanoic acid did not alter 10 μM ADP-induced primary aggregation relative to the aggregation induced in the presence of aspirin alone.

Representative samples of the 13-azaprostanoic acid derivatives and *cis*-15-azaprostanoic acid were also tested for their effect as inhibitors of prostaglandin synthetase (cyclooxygenase). The basis of the assay is the measurement of the arachidonic acid dependent formation of adrenochrome from L-epinephrine during prostaglandin biosynthesis.[36] The results of these experiments are shown in Figure 1. A concentration of 5×10^{-4} M of the *cis*- and *trans*-13-azaprostanoic acids **4b** and **4a**, respectively, the *trans*-13-aza-20-norprostanoic acid analogue **4e**, and the *trans*-benzyl derivative **4g** did not significantly inhibit cyclooxygenase activity. Likewise, *cis*-15-azaprostanoic acid (**11a**) failed to inhibit the enzyme at 1×10^{-4} M. Weak inhibitory activity on the order of 30% was observed with the *cis*- and *trans*-13-aza-20-homo analogues **4d** and **4c**, respectively. Under these assay conditions, 100% inhibition of enzyme activity was produced by indomethacin at 2×10^{-5} M.

The preliminary biological screening of the azaprostanoic acids synthesized during this investigation has therefore revealed that several of the derivatives, most notably *trans*-13-azaprostanoic acid (**4a**), are potent inhibitors of platelet aggregation. As might be expected by the similarity of these compounds to the prostaglandins, their inhibitory activity in the platelet is specific to the arachidonic acid cascade. This is borne out by the observation that none of the derivatives tested had inhibitory activity on the primary, reversible phase of ADP-induced aggregation. In addition, this indicates that the azaprostanoic acids do not act on the platelet by a nonspecific effect, such as deformation of the platelet membrane.

The ability of the azaprostanoates to selectively inhibit arachidonic acid induced platelet aggregation led us to postulate that these compounds might be acting at one of three distinctly different sites. First, the azaprostanoic acids might be inhibitors of the cyclooxygenase enzyme complex which transforms the fatty acid precursors to the prostaglandin endoperoxides PGG_2 and PGH_2. In this regard, they would have a mode of action similar to the nonsteroidal antiinflammatory agents such as aspirin and indomethacin.[6,7] A second potential site of action might be the inhibition of thromboxane synthetase, thereby

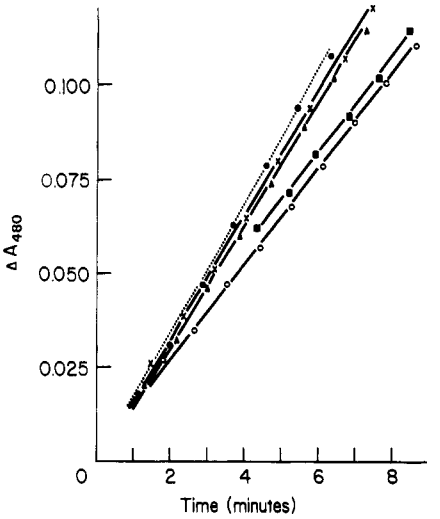


Figure 1. Effect of azaprostanoic acids (5×10^{-4} M) on prostaglandin cyclooxygenase activity from beef seminal vesicles by the method of Sih.[36] Representative examples: control (··●··), *cis*- (–x–; **4a**) and *trans*-13-azaprostanoic acids (–▲–; **4b**), *cis*- (–■–; **4d**) and *trans*-20-homo-13-azaprostanoic acids (–○–; **4e**). Indomethacin (2×10^{-5} M) showed $\Delta A_{480} = 0$ for 0–8 min.

blocking the transformation of the endoperoxides to the proaggregatory agent TXA_2, similar to the action of imidazole and the Azo I analogue.[19] The third possible site of action could be the direct inhibition of arachidonic acid metabolites at their corresponding receptor site(s).

In order to check the possibility that the azaprostanoic acids act as inhibitors of the cyclooxygenase enzyme, we tested the analogues in a standard prostaglandin biosynthesis assay. Although this assay is an indirect measurement of cyclooxygenase activity, it offers the advantage that total enzyme activity is measured and, consequently, is not limited to the measurement of any one product of the biotransformation.[36] The azaprostanoic acids were found to have very little or no effect on prostaglandin synthetase derived from bovine seminal vesicles. The most potent inhibitor of the platelet, i.e., *trans*-13-azaprostanoic acid (**4a**), had less than 10% inhibitory activity on the cyclooxygenase at a concentration one order of magnitude greater than that necessary for 100% inhibition of platelet aggregation. At the highest concentration tested in this assay system (5×10^{-4} M), only the *cis*- and *trans*-20-homo analogues showed significant inhibitory activity (approximately 30%).

Although our assay employed a cyclooxygenase preparation derived from bovine seminal vesicles, there is considerable evidence to suggest that the enzyme complex from bovine seminal vesicles and human platelets are similar.[37] We have therefore concluded that the inhibitory activity of the azaprostanoic acids on platelet aggregation is not caused by inhibition of the platelet cyclooxygenase enzyme system and thus resides beyond the synthesis of the endoperoxides. A detailed study, which will be reported elsewhere, indicates that the title compounds do not block TXA_2 synthetase and that they are likely acting as direct TXA_2 antagonists, presumably at the receptor level.[38]

The inhibitory effect of the 13-azaprostanoic acids on arachidonate-induced platelet aggregation was also found to be highly sensitive to stereochemical configuration as well as to the length of the amino acid side chain. Thus, all of the cis isomers and the 20-nor and 20-homo analogues, regardless of stereochemistry, show decreased activity when compared with the aza analogue of the natural prostaglandin skeletal arrangement.

The unique pharmacological activity exhibited by the azaprostanoates clearly suggests many uses as biological probes to further elucidate the role of the arachidonic acid cascade in platelet aggregation. In addition, the potential involvement of thromboxanes in a number of disease states, such as thromboembolism and hypertension, further suggests that the title compounds may well represent new therapeutic leads in such areas.

Experimental Section

All melting points were determined in capillary tubes on a Thomas-Hoover melting point apparatus and are uncorrected. NMR spectra were recorded by means of a Varian T-60A spectrometer equipped with a Nicolet TT-7 Fourier transform accessory and ^{13}C probe. Chemical shifts are reported in parts per million (δ) downfield from internal $(CH_3)_4Si$. Mass spectra were obtained by Mr. Richard Dvorak at 70 eV using a Hitachi Perkin-Elmer RMU-6D single-focusing mass spectrometer and are reported as m/e (relative intensity). IR spectra were obtained on a Perkin-Elmer 337 recording infrared spectrophotometer. The structures of all compounds were confirmed by spectroscopic analysis. Microanalyses were performed by Micro-Tech Laboratories, Skokie, Ill., and results are indicated by symbols of the elements and are within ±0.4% of theory. Thin-layer chromatographs (TLC) were developed on 10-cm slides coated with silica gel and a fluorescent indicator (Eastman Chromagram Sheet 6060). Spots were visualized by UV light, iodine vapor, or nitroprusside–acetaldehyde spray reagent,[39] where appropriate. Reaction mixtures were routinely analyzed by gas–liquid chromatography (GLC) using a Hewlett-Packard 5750 research chromatograph fitted with a 10-ft, 3% SE-30 analytical column ($^1/_8$ in. o.d.). Preparative gas chromatography employed a 20-ft, 5% SE-30 column (0.5 in. o.d.).

2-(6-Carbomethoxyhexyl)cyclopentanone (3). To 2-(6-carboxyhexyl)cyclopentanone[22] (3.30 g, 15.6 mmol) in anhydrous methanol (60 mL) was added 0.25 g of *p*-toluenesulfonic acid, and the mixture was stirred overnight at 25 °C. Excess methanol was evaporated in vacuo, the residue was dissolved in ether, and the solution was washed one time with ice-cold 5% aqueous $NaHCO_3$ and one time with water, dried (K_2CO_3), filtered, and concentrated to give the keto ester **3** (3.27 g, 93%). An analytical sample was obtained by preparative GLC: ^1H NMR (CCl_4) δ 3.67 (s, 3 H, CO_2CH_3), 2.6–1.0 (complex m, 19 H); MS 226 (6), 195 (8), 194 (5), 143 (6), 111 (8), 97 (9), 84 (100), 83 (17). Anal. ($C_{13}H_{22}O_2$) C, H.

***cis*-Amino Acids 4 (Table II). Typical Procedure. *cis*-13-Azaprostanoic Acid (4b).** To a solution of 2-(6-carbomethoxyhexyl)cyclopentanone (**3**; 2.0 g, 8.8 mmol) in 45 mL of absolute methanol containing approximately 2 g of molecular sieves (3 Å) was added dropwise *n*-heptylamine (2.04 g, 17.7 mmol). The solution was stirred under a nitrogen atmosphere at room temperature for 2 days. After cooling the solution (ice bath), 98% sodium borohydride (0.36 g, 9.3 mmol) was added, and the mixture was stirred for 30 min. The excess borohydride was decomposed by the addition of 1 mL of acetone. The solvents were removed in vacuo, and the residue was dissolved in ether, washed twice with saturated NaCl and once with water, dried over potassium carbonate, filtered, and concentrated to give a yellow oil. Column chromatography of this oil on 75 g of silica gel with ether–hexane (62.5:37.5) gave *cis*-13-azaprostanoic acid methyl ester (790 mg, 28%): R_f (ether) 0.52. The ester (790 mg, 2.35 mmol) was boiled with stirring in 15 mL of 2.5% aqueous sodium hydroxide until homogeneous (~4 h). After cooling, the solution was acidified by the dropwise addition of 5% aqueous hydrochloric acid, then made strongly basic by addition of excess concentrated ammonium hydroxide, and heated gently to expel excess ammonia. The crude amino acid was collected by filtration, washed with water, and air-dried to give the *cis*-13-azaprostanoic acid **4b**: yield 498 mg (66%); mp 98.5–103.5 °C. Three recrystallizations from ethanol–water gave the analytical sample: mp 107.5–109.5 °C; MS 311 (10), 226 (100), 154 (64); TLC R_f as methyl ester (ether) 0.61; NMR 3.20–2.80 (C*H*NH–) ppm.[40] Anal. ($C_{19}H_{37}NO_2$) C, H, N.

Compound 4d: yield 35 and 97%;[41] mp (ethanol–water) 86.5–87.5 °C; MS 325 (10), 226 (100), 168 (56); TLC R_f as methyl ester (ether) 0.52; NMR 3.10–2.70 (>C*H*NH–).[40] Anal. ($C_{20}H_{39}NO_2$) C, H, N.

Compound 4f: yield 35 and 41%;[41] mp (ethanol–water) 86.5–87.5 °C; MS 297 (8), 226 (100), 140 (88); TLC R_f as methyl ester (ether) 0.55; NMR 3.10–2.75 (>C*H*NH–) ppm.[40] Anal. ($C_{18}H_{35}NO_2$) C, H, N.

Compound 4h: yield 21 and 80%;[41] mp (ethanol–water) 129–130 °C; MS 303 (9), 146 (87), 91 (100); TLC R_f as methyl ester (ether) 0.57; NMR 3.20–2.85 (>C*H*NH–) ppm.[40] Anal. ($C_{19}H_{29}NO_2$) C, H, N.

***trans*-Amino Acids 4 (Table II). Typical Procedure. *trans*-13-Azaprostanoic Acid (4a).** Further elution of the column prepared for the methyl ester of the cis isomer **4b** with ether–hexane (62.5:37.5) gave *trans*-13-azaprostanoic acid methyl ester (901 mg, 32%). Hydrolysis and workup of the *trans*-methyl ester (901 mg, 2.8 mmol) by the usual method gave the amino acid **4a**: yield 547 mg (63%); mp 112–114.5 °C. Two recrystallizations from ethanol–water gave the analytical sample: mp 115.5–116 °C; MS 311 (10), 226 (100), 154 (67); TLC R_f as methyl ester (ether) 0.49; NMR 2.80–2.45 (C*H*NH–) ppm.[40] Anal. ($C_{19}H_{37}NO_2$) C, H, N.

Compound 4c: yield 28 and 83%;[41] mp (ethanol–water) 110.5–111.5 °C; MS 325 (10), 226 (100), 168 (61); TLC R_f as methyl ester (ether) 0.40; NMR 2.75–2.45 (>C*H*NH–) ppm.[40] Anal. ($C_{20}H_{39}NO_2$) C, H, N.

Compound 4e: yield 31 and 70%;[41] mp (ethanol–water) 115–115.5 °C; MS 297 (9), 226 (100), 140 (87); TLC R_f as methyl ester (ether) 0.46; NMR 2.75–2.46 (>C*H*NH–) ppm.[40] Anal. ($C_{18}H_{35}NO_2$) C, H, N.

Compound 4g: yield 46 and 89%;[41] mp (ethanol) 151–151.5 °C; MS 303 (8), 146 (75), 91 (100); TLC R_f as methyl ester (ether) 0.44; NMR 2.85–2.45 (>C*H*NH–) ppm.[40] Anal. ($C_{19}H_{29}NO_2$) C, H, N.

***cis*-2-(*N*-Benzylamino)-1-methylcyclopentane (5a).** Following the procedure used for the synthesis of the azaprostanoic acids 4, 2-methylcyclopentanone (1.00 g, 10 mmol) and benzylamine (2.22 g, 20 mmol) gave 2.98 g of yellow oil. Column chromatography of 546 mg of the oil on 80 g of silica gel with ether–hexane, 1:1, gave the *cis*-benzylamine **5a** (131 mg, 38%): TLC R_f 0.40 (ether–hexane, 1:1); ^1H NMR (CCl_4) δ 3.2–2.8 (m, 1 H, C*H*N); MS 189 (40), 160 (17), 146 (79), 132 (12), 91 (100). The hydrochloride salt was prepared by bubbling dry HCl gas into an ethereal solution of the amine. The salt was collected by filtration and washed with ether. Two recrystallizations from ethanol–ether gave the analytical sample, mp 153–154 °C. Anal. ($C_{13}H_{20}NCl$) C, H, N.

***trans*-2-(*N*-Benzylamino)-1-methylcyclopentane (5b).** Further elution of the column used for the isolation of the cis isomer **5a** with ether–hexane, 1:1, gave the *trans*-benzylamine **5b** (124 mg, 36%): TLC R_f 0.56 (ether); ^1H NMR (CCl_4) δ 2.8–2.3 (m, 1 H, C*H*N); MS 189 (44), 160 (17), 146 (100), 132 (13), 91 (100). The hydrochloride salt was prepared in the same manner as that for the cis isomer **5a**. One recrystallization from ethanol–ether gave the analytical sample, mp 177–178 °C. Anal. ($C_{13}H_{20}NCl$) C, H, N.

***trans*-2-(*N*-Benzylamino)-1-methylcyclopentane (5b). From *trans*-2-Methylcyclopentylamine.** To a solution of *trans*-*N*-(2-methylcyclopentyl)benzamide[30] (0.38 g, 1.9 mmol) in 20 mL of dry benzene was added dropwise 2.5 mL of Red-Al® (70% in benzene) with stirring. The mixture was refluxed overnight. After cooling the mixture, 10 mL of water was added, and stirring was continued for 1 h. The solution was extracted with benzene, and the combined extracts were dried (K_2CO_3), filtered, and concentrated in vacuo to give the *trans*-benzylamine **5b** (0.29 g, 80%). The chromatographic and spectroscopic properties of this product, as well as the melting point of the hydrochloride salt, were identical with those obtained for the compound prepared by the alternate synthetic route.

***cis*-1-Methyl-2-[2-(*N*-cyclohexylamino)ethyl]cyclopentane (8a).**[42] To a suspension of 50% sodium hydride (0.42 g, 8.8 mmol) in 5 mL of dry THF under a nitrogen atmosphere at 0 °C was added diethyl 2-(cyclohexylimino)ethylphosphonate[32] (2.25 g, 8.6 mmol) in 15 mL of THF. After stirring the mixture for 15 min, 2-methylcyclopentanone (0.70 g, 7.1 mmol) in 7 mL of THF was added, and the mixture was stirred for 3 h at room temperature. The solution was poured onto crushed ice and extracted three times with ether. The combined extracts were dried (Na_2SO_4), filtered, and concentrated to give 1.86 g of a dark yellow oil

containing the α,β-unsaturated aldimine and excess phosphonate. The oil was dissolved in 200 mL of absolute ethanol, to which was added 200 mg of 5% palladium on charcoal. The solution was shaken for 3 h at 25 °C on a Parr hydrogenator under a hydrogen pressure of 40 psi. After filtration and concentration in vacuo, 1.68 g of a pale yellow oil was obtained. Analysis of the oil by GC/MC indicated a 4:1 mixture of cis and trans isomers. The mixture of amines was characterized as the HCl salt, mp 187.5–188 °C. Anal. ($C_{14}H_{28}NCl$) C, H, N. A portion of the HCl salt was neutralized with dilute $NaHCO_3$ and extracted with ether. The ether layer was dried (Na_2SO_4) and the solvents were removed, giving a colorless oil consisting solely of a 4:1 mixture of the *cis*- and *trans*-amines **8**. The isomers were separated by preparative GLC with the major cis isomer **8a** eluting first. Anal. ($C_{14}H_{17}N$) C, H, N.

***trans*-1-Methyl-2-[2-(*N*-cyclohexylamino)ethyl]cyclopentane (8b).**[42] Further elution of the GLC column used for the preparation of the cis isomer **8a** gave the trans derivative **8b**, characterized as its HCl salt: mp (methanol–ether) 190–192 °C. Anal. ($C_{14}H_{28}NCl$) C, H, N.

1-*n*-Propyl-2-[2-(*N*-cyclohexylamino)ethyl]cyclopentane (9). By the same procedure used for the synthesis of **8a**, diethyl 2-(cyclohexylimino)ethylphosphonate[32] (2.50 g, 9.6 mmol), 50% sodium hydride (0.46 g, 9.4 mmol), and 2-allylcyclopentanone[33] (1.0 g, 8.1 mmol) were stirred in THF under nitrogen for 5 h at room temperature and worked up to give 2.39 g of dark yellow oil. Catalytic reduction of this oil was carried out in 175 mL of 95% ethanol with 200 mg of 5% palladium on charcoal at 40 psi hydrogen pressure for 2 h. After filtration and concentration, the residue was dissolved in ether, and when extraction with 10% aqueous hydrochloric acid was attempted a water-insoluble precipitate formed in the ether layer. The white precipitate was collected by filtration, washed with ether, and dried to give the amine (stereochemistry undetermined) as its HCl salt (1.46 g, 66%). Two recrystallizations from ethanol–ether gave the analytical sample: mp 182–184 °C, MS 237 (8), 194 (25), 166 (5), 112 (100). Anal. ($C_{16}H_{32}NCl$) C, H, N.

2-Carbethoxy-2-*n*-heptylcyclopentanone (12). To a solution of 2-carbethoxycyclopentanone (10.0 g, 64 mmol) and 1-bromoheptane (14.8 g, 83 mmol) in 130 mL of dry acetone was added anhydrous K_2CO_3 (23.8 g, 172 mmol). The mixture was heated at reflux with stirring for 3 days. After cooling the mixture, the solution was filtered and concentrated to give 19.6 g yellow oil. The oil was heated in vacuo to remove low-boiling impurities (79–90 °C, 35 mmHg) and the residue obtained was nearly pure **12** (14.99 g, 92%) as judged by GLC. An analytical sample was obtained by preparative GLC: ^{1}H NMR (CCl_4) δ 4.10 (q, J = 7 Hz, 2 H, $CO_2CH_2CH_3$), 1.25 (t, J = 7 Hz, 3 H, $CO_2CH_2CH_3$); MS 254 (1), 209 (6), 156 (100), 110 (26). Anal. ($C_{15}H_{26}O_3$) C, H.

2-*n*-Heptylcyclopentanone (13). The keto ester **12** (8.54 g, 33 mmol) was heated to reflux in 90 mL of 2:1 water–concentrated H_2SO_4 and 25 mL of 95% ethanol for 42 h. The solution was cooled and extracted with ether. The combined extracts were dried (Na_2SO_4), filtered, and concentrated to an oil, which gave, after distillation (80 °C, 0.01 mmHg), ketone **13**: yield 5.79 g (95%); homogeneous to GLC; IR (neat) 1745 cm^{-1} (C=O); MS 182 (8), 97 (16), 84 (100), 55 (12), 41 (11). Anal. ($C_{12}H_{22}O$) C, H.

1-*n*-Heptyl-2-[2-(*N*-cyclohexylamino)ethyl]cyclopentane (10). By the same procedure used for the synthesis of **8a**, diethyl 2-(cyclohexylimino)ethylphosphonate[32] (2.58 g, 9.9 mmol), 50% sodium hydride (0.48 g, 9.9 mmol) and ketone **13** (1.50 g, 8.2 mmol) were stirred in THF under nitrogen for 4 h at room temperature and worked up in the aforementioned manner to give 3.07 g of yellow oil. Catalytic reduction of this oil was carried out in 200 mL of 95% ethanol with 200 mg of 5% palladium on charcoal at 40 psi hydrogen pressure for 2 h. Filtration and concentration in vacuo gave 2.89 g of pale yellow oil. GLC analysis indicated this oil contained greater than 90% of the expected product **10**. An analytical sample was obtained by preparative GLC: MS 293 (4), 250 (10), 222 (5), 210 (4), 112 (100). Anal. ($C_{20}H_{39}N$) C, H, N.

Diethyl 2-(*n*-Pentylimino)ethylphosphonate (7b). To a solution of diethyl formylmethylphosphonate[32] (8.1 g, 45 mmol) in 50 mL of absolute methanol at ice-bath temperature under a nitrogen atmosphere was added dropwise *n*-pentylamine (3.9 g, 45 mmol). The mixture was stirred at room temperature for 20 min. After removal of the solvent in vacuo, the residue was dissolved in ether, dried (K_2CO_3), filtered, and concentrated to give 9.5 g of yellow oil. Short-path distillation of K_2CO_3 (ca. 200 mg) gave phosphonate **10** (6.0 g, 54% isolated): IR (neat) 3270 (br, NH), 1620 cm^{-1} ($-CH_2CH{=}N-$); ^{1}H NMR (CCl_4) δ 7.40–6.20 (m, 2 H, $-CH{=}CH-$), 4.30–3.60 (m, 4 H, $-OCH_2CH_3$), 1.25 (t, J = 7 Hz, $-OCH_2CH_3$); MS 249 (31), 220 (24), 206 (23), 192 (100), 165 (23), 164 (30), 152 (4), 136 (37), 118 (23), 112 (27), 111 (25), 98 (33). Anal. ($C_{11}H_{24}NO_3P$) C, H.

***cis*-15-Azaprostanoic Acid (11a).** To lithium hydride (0.124 g, 15 mmol) in 3 mL of THF was added dropwise a solution of phosphonate **7b** (3.81 g, 15 mmol) in 14 mL of THF. The solution was gently heated (70–80 °C) with stirring under a nitrogen atmosphere overnight to generate the carbanion. After cooling the solution, a solution of keto ester **3** (1.54 g, 6.8 mmol) in 8 mL of THF was added and stirred at room temperature under nitrogen for 9 h. The mixture was poured onto crushed ice and extracted with ether. The combined extracts were dried (K_2CO_3), filtered, and concentrated to give 4.67 g of a yellow oil. The oil was dissolved in 200 mL of 95% ethanol to which was added 200 mg of 5% palladium on charcoal, and the solution was shaken for 3.5 h at 40 psi hydrogen pressure. Filtration and concentration gave 4.17 g of yellow oil. To this oil was added 60 mL of 2.5% aqueous NaOH, and the mixture was boiled with stirring until a homogeneous solution was obtained (2 h). After cooling the mixture, 5% HCl was added dropwise until acidic and then concentrated ammonium hydroxide was added until strongly basic. The mixture was heated gently with stirring to expel excess ammonia and a dark orange oil separated out of solution. The solution was extracted several times with octanol, and the combined extracts were concentrated in vacuo with gentle heating (40–70 °C) to give 3.75 g of dark yellow oil. Column chromatography of 0.99 g of the oil on 70 g of silica gel and elution with $CHCl_3$–MeOH–NH_4OH, 94:5:1, gave the major cis isomer **11a** (203 mg, 36%). Three recrystallizations from ethanol–water gave the analytical sample: mp 107–107.5 °C; MS 311 (6), 254 (30), 100 (100). Anal. ($C_{19}H_{37}NO_2$) C, H, N. Further elution of the above column gave a small amount of the trans isomer whose mass spectrum was identical with that obtained for the major isomer. This latter product was clearly different from the major isomer when subjected to TLC.

Biological Methods. The assay for prostaglandin synthetase (cyclooxygenase) activity was performed by the method of Takeguchi and Sih[36] with only minor modification. Each assay contained 6.7 mg/mL of a crude, freeze-dried powder of prostaglandin synthetase from beef seminal vesicles, purchased from Miles Research Products. The enzyme was solubilized with 0.05% Triton X-100. Arachidonic acid and L-epinephrine (high-purity grades) were purchased from Sigma Chemical Co.

Platelet aggregation was studied by the turbidometric method of Born[43] at 37 °C over a 3-min time course. Citrated human platelet-rich plasma from normal, healthy donors who had not ingested aspirin in 48 h was purchased from a commercial blood bank. The plasma was centrifuged at 164*g* to remove any remaining red blood cells and maintained at 25 °C until the experiments were performed. The reagents were added as 10 μL aliquots per 1 mL of platelet-rich plasma to give the specified concentration (see Pharmacology Section).

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Acute Effects of Alkylating Agents on Canine Renal Function. 1. [4-(2-Bromoalkanoyl)phenoxy]acetic Acids

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A group of [4-(2-bromoalkanoyl)phenoxy]acetic acids was studied to determine if there was an association between the alkylating ability and the diuretic activity of its members. Acute studies in dogs revealed that there is not a consistent correlation in the alkylating potential of these α-bromo ketones and their ability to induce a diuretic response. In addition, pretreatment of dogs with the various α-bromo ketones did not alter the diuretic activity normally observed with ethacrynic acid (EA). The role of chemical-induced renal tissue alkylation in the initiation of a diuresis or a nephrotoxic response is discussed.

Ethacrynic acid (EA), a potent diuretic agent, is known to alkylate various nucleophiles in vitro by a Michael-type reaction, and there is no reason to doubt that a similar alkylation reaction might occur in vivo.[1-4] In fact, it has been suggested that the alkylation of as yet undetermined renal tissue components by EA is responsible for initiating a diuretic response.[1,3-6] However, recent information has emerged that challenges the necessity of EA-induced renal tissue alkylation for the expression of a diuretic response. The finding that certain EA-related compounds possess potent diuretic and uricosuric properties but lack the capability of alkylating nucleophiles has led Cragoe et al.[7] and Woltersdorf et al.[8] to conclude that EA and EA-related compounds probably induce a diuresis by interacting with renal tissue components by two mechanisms: (1) an alkylation reaction which contributes only to a minor extent to the induction of a diuresis and (2) a nonalkylating type of interaction which is of major importance in triggering a diuresis.

To examine further the relative importance of renal tissue alkylation by EA and EA-related compounds for the expression of a diuretic response, we designed, synthesized, and evaluated the renal effects of a group of EA-related α-bromo ketones (i.e., [4-(2-bromoalkanoyl)phenoxy]acetic acids) which not only vary greatly in their potential alkylating ability but, in addition, alkylate nucleophiles by an S_N2 reaction rather than by a Michael-type reaction. It has been firmly established that S_N2 reactions proceed much more readily with primary α-halo ketones than with secondary and tertiary α-halo ketones.[9] Thus, to attain derivatives with varying degrees of alkylating ability, we synthesized **2a-c** from the corresponding ketones. The diuretic activity of a group of chemically related α-halo ketones has been reported in dogs; however, no mention was made of the dose administered or the nature of the time-action curve.[10,11]

It might be anticipated that the in vitro, as well as the in vivo, alkylating ability of the three α-bromo ketones studied herein would follow the order **2c > 2b > 2a**. If renal tissue alkylation is a prerequisite for the expression of a diuretic response with EA and EA-related compounds, then the diuretic activity associated with **2a-c** should

0022-2623/79/1822-0830$01.00/0