(1.95 g, 14.6 mmol) in Et_2O (100 mL) was cooled in an ice bath, and $LiAlH_4$ (1.66 g, 43.9 mmol) was added. After the mixture was stirred for 30 min, a solution of **24f** (7.1 g, 14.6 mmol) in Et_2O (250 mL) was added, and the mixture was refluxed for 18 h. The cooled mixture was treated sequentially dropwise with H_2O (1.7 mL), 15% NaOH (1.7 mL), and H_2O (5.1 mL) and then filtered through Celite. The filtrate was diluted with EtOAc and washed with H_2O. Evaporation gave 5.8 g of a glass, which was chromatographed to give 4.43 g (62%) of **26Pf** as a glass. A portion of this material was converted to the HCl salt, which was obtained as white crystals, mp 118–122 °C, from EtOAc. Several recrystallizations from EtOAc gave analytically pure material with an indefinite melting point. Anal. ($C_{32}H_{41}NO_3$·HCl) C, H, N. In a similar manner, **26Bc** was obtained as a foam in 84% yield. Crystals of the HCl salt, mp 169–171 °C, were obtained from EtOAc. Anal. ($C_{31}H_{39}NO_3$·HCl) C, H, N. Compound **26Bf** was obtained in 65% yield and crystallized as the HCl salt: mp sinters at 116 °C, melts at 123–130 °C. Anal. ($C_{33}H_{43}NO_3$·HCl) C, H, N.

7β-(Arylalkyl)-17-(cycloalkylmethyl)-4,5α-epoxy-3-methoxy-7α-methylmorphinan-6-ones (27P,B). To a solution of Me_2SO (1.0 mL, 14.3 mmol) in CH_2Cl_2 under argon at −60 °C was added slowly, dropwise, trifluoroacetic anhydride (1.5 mL, 10.7 mmol) in CH_2Cl_2 (7 mL). After 10 min, a solution of **26Pf** (3.48 g, 7.1 mmol) in CH_2Cl_2 (50 mL) was added slowly. The mixture was kept at −60 °C for 90 min, then TEA (3 mL) was added, and the mixture was warmed to room temperature. After the mixture was washed with H_2O, evaporation of the organic phase gave 3.47 g of **27Pf** as a foam. The HCl salt, mp 105–110 °C, was recrystallized several times from EtOAc and best analyzed as containing 0.33 mol of EtOAc. Anal. ($C_{32}H_{39}NO_3$·HCl·0.33EtOAc) C, H, N. The free base of **27Bc** was obtained as crystals, mp 150–151 °C, in 77% yield after chromatography and crystallization from EtOAc–Et_2O. Anal. ($C_{31}H_{37}NO_3$) C, H, N. Compound **27Bf** was obtained in quantitative yield as crystals, mp 149–152 °C. Recrystallization from EtOAc gave a 96% yield of pure **27Bf**, mp 150–151.5 °C. Anal. ($C_{33}H_{41}NO_3$) C, H, N.

7β-(Arylalkyl)-17-(cycloalkylmethyl)-4,5α-epoxy-3-hydroxy-7α-methylmorphinan-6-ones (28P,B). A mixture of **27** and 48% HBr was refluxed for 15 min and processed as described previously. Chromatography gave **28Pf** in 63% yield as a foam. Crystals of **28Pf**, mp 183.5–185 °C, were deposited from EtOAc. Anal. ($C_{31}H_{37}NO_3$) C, H, N. Compound **28Bc** was obtained in 42% yield after chromatography. The HCl salt, mp sinters above 200 °C, crystallized from EtOAc. Anal. ($C_{30}H_{35}NO_3$·HCl) C, H, N. The foam obtained upon workup of **28Bf** crystallized upon titration with EtOAc. These crystals, obtained in 53% yield, were recrystallized from EtOAc to give pure **28Bf**, mp 186–188.5 °C. Anal. ($C_{32}H_{39}NO_3$) C, H, N.

Registry No. **3**, 85455-22-7; **5a**, 85534-09-4; **5b**, 85552-56-3; **5c**, 85534-10-7; **5d**, 85534-11-8; **5d**·HCl, 85534-12-9; **5g**, 85534-13-0; **5h**, 85534-14-1; **5h**·HCl, 85534-15-2; **6**, 85534-16-3; **7a**, 85534-17-4; **7a**·HCl, 85534-18-5; **7b**, 85534-19-6; **7b**·HCl, 85534-20-9; **7c**, 85534-21-0; **7d**, 85534-23-2; **7d**·HCl, 85534-22-1; **7f**, 85455-23-8; **7g**, 85534-25-4; **7g**·HCl, 85534-24-3; **7h**, 85534-26-5; **8b**, 85534-27-6; **8c**, 85534-28-7; **8d**, 85534-29-8; **8f**, 85455-24-9; **8g**, 85434-31-7; **8g**·HCl, 85534-30-1; **8h**, 85534-33-4; **8h**·HCl, 85534-32-3; **10c**, 85534-34-5; **10d**, 85534-35-6; **10f**, 85534-36-7; **10g**, 85534-37-8; **11Bc**, 85552-58-5; **11Bc**·HCl, 85552-57-4; **11Bd**, 85534-45-8; **11Bf**, 85534-47-0; **11Bf**·HCl, 85534-46-9; **11Bg**, 85534-49-2; **11Bg**·HCl, 85534-48-1; **11Pc**, 85534-39-0; **11Pc**·HCl, 85534-38-9; **11Pd**, 85534-40-3; **11Pf**, 85534-42-5; **11Pf**·HCl, 85534-41-4; **11Pg**, 85534-44-7; **11Pg**·HCl, 85534-43-6; **12Bc**, 85534-56-1; **12Bd**, 85534-58-3; **12Bd**·HCl, 85534-57-2; **12Bf**, 85534-60-7; **12Bf**·HCl, 85534-59-4; **12Bg**, 85534-62-9; **12Bg**·HCl, 85534-61-8; **12Pc**, 85534-50-5; **12Pd**, 85534-52-7; **12Pd**·HCl, 85534-51-6; **12Pf**, 85534-53-8; **12Pg**, 85534-55-0; **12Pg**·HCl, 85534-54-9; **16**, 85455-05-6; (*E*)-**17c**, 85534-63-0; (*Z*)-**17c**, 85534-64-1; **17d**, 85534-65-2; **17f**, 85534-66-3; **17g**, 85534-67-4; **18c**, 85534-68-5; **18c** *d*-tartrate, 85534-69-6; **18d**, 85534-70-9; **18d** *d*-tartrate, 85534-71-0; **18f**, 85455-07-8; **18g**, 85534-72-1; **18g** *d*-tartrate, 85534-73-2; **19** (isomer 1), 85534-74-3; **19** (isomer 2), 85534-75-4; **20c**, 85534-76-5; **20d**, 85534-77-6; **20g**, 85552-59-6; **21c**, 85534-78-7; **21c** *d*-hemitartrate, 85534-79-8; **21d**, 85534-80-1; **21d** *d*-tartrate, 85534-81-2; **21f**, 85455-08-9; **21g**, 85534-82-3; **21g** *d*-tartrate, 85534-83-4; **22c**, 85534-84-5; **22d**, 85534-85-6; **22f**, 85534-86-7; **23c**, 85534-87-8; **23d**, 85534-88-9; **23f**, 85534-89-0; **24Bc**, 85534-90-3; **24Bd**, 85534-91-4; **24Bf**, 85534-92-5; **24Pc**, 85534-93-6; **24Pd**, 85534-94-7; **24Pf**, 85534-95-8; **25Bc**, 85534-96-9; **25Bd**, 85534-97-0; **25Bf**, 85534-98-1; **25Pc**, 85534-99-2; **25Pd**, 85535-00-8; **25Pf**, 85535-01-9; **26Bc**, 85535-02-0; **26Bc**·HCl, 85535-03-1; **26Bf**, 85535-04-2; **26Bf**·HCl, 85535-05-3; **26Pf**, 85535-06-4; **26Pf**·HCl, 85535-07-5; **27Bc**, 85535-08-6; **27Bf**, 85535-09-7; **27Pf**, 85535-10-0; **27Pf**·HCl, 85535-11-1; **28Bc**, 85535-12-2; **28Bc**·HCl, 85535-13-3; **28Bf**, 85535-14-4; **28Pf**, 85535-15-5; $(C_6H_5)_3PCH_3Br$, 1779-49-3; $(C_6H_5)_3P(CH_2)_2CH_3Br$, 6228-47-3; $(C_6H_5)_3PCH_2C_6H_5Br$, 1449-46-3; $(C_6H_5)_3P(CH_2)_2C_6H_5Br$, 53213-26-6; $(C_6H_5)_3P(CH_2)_4C_6H_5Br$, 37748-19-9; $(C_6H_5)_3P(CH_2)_5C_6H_5Br$, 17483-25-9; phenyllithium, 591-51-5.

2-(6-Carboxyhexyl)cyclopentanone Hexylhydrazone. A Potent and Time-Dependent Inhibitor of Platelet Aggregation

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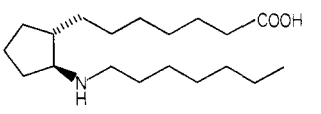
Two new azaprostanoids, a hydrazone (**3**) and hydrazide (**4**), have been prepared by the condensation of 2-(6-carboxyhexyl)cyclopentanone with *n*-hexylhydrazine and caproic acid hydrazide. Preliminary results with the stable hydrazide **4** indicate that it inhibits arachidonic acid (AA) induced human platelet aggregation and that, unlike 13-azaprostanoic acid (**1**), its site of action is at the cyclooxygenase level. Results with the unstable hydrazone derivative **3** indicate it to be a potent and time-dependent inhibitor of AA-induced human platelet aggregation, with its site of action also at the cyclooxygenase level.

In a continued search for modulators of platelet prostaglandin action, we prepared the azaprostanoid derivatives **3** and **4**. The rational for the synthesis of these compounds was based on our previous observations of antiplatelet activity for the simple *trans*-13-azaprostanoic acid (**1**).[1] This derivative appears to act as a direct thromboxane antagonist at the receptor level.[2] We



1

speculated that the nonbonding electrons of nitrogen in the 13-azaprostanoic acid might be mimicking the π system

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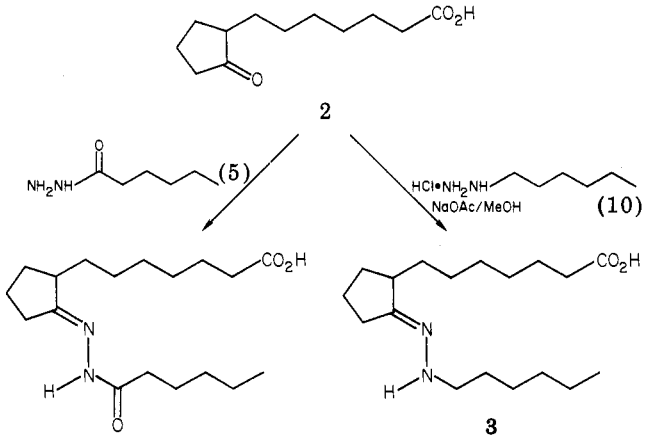
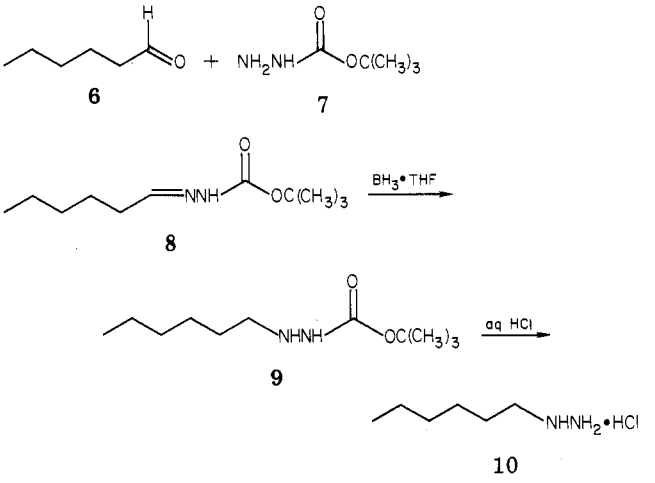
(1) Venton, D. L.; Enke, S. E.; Le Breton, G. C. *J. Med. Chem.* **1972**, *22*(7), 824.

0022-2623/83/1826-1056$01.50/0

Scheme I



Scheme II



of the 13,14 double bond in the natural prostaglandin thromboxane A_2 (TXA_2) substrate and that compounds containing nitrogens at both the 13 and 14 positions might show enhanced biological activity.

Chemistry. The stable hydrazide derivative **4** was prepared as shown in Scheme I. The required caproic acid hydrazide **5** was prepared from the corresponding methyl ester and hydrazine in good yield. Condensation of the hydrazide side chain with the keto acid **2**[3] in methanol gave 2-(6-carboxyhexyl)cyclopentylidenecaproic acid hydrazide (**4**) in good yield. The hydrazide **4** can exist in two possible isomeric states about the carbon–nitrogen double bond. Based on steric considerations, the configuration shown in Scheme I where the nitrogen–nitrogen single bond is anti to the upper side chain would seem to be the most stable isomer. Although we do not have direct evidence for any particular configuration, ^{13}C NMR shows 18 carbon transitions, suggesting that the derivative is not a mixture of the two possible isomers.

The lower side-chain **10**, for the synthesis of the hydrazone derivative **3** (see Scheme I), was prepared by a new high-yielding synthesis of monosubstituted hydrazines recently developed in our laboratories (Scheme II).[4] Condensation of the *n*-hexylhydrazine hydrochloride (**10**) with the keto acid **2** in methanol, buffered with sodium acetate, gave the unstable 2-(6-carboxyhexyl)cyclopentanone hexylhydrazone (**3**). The product is ether soluble and can be washed free of salts with water. Removal of the solvent gives a slightly yellow, crystalline solid, which shows only minor impurities by TLC. Recrystallization from ether–pentane at low temperature gives chromatographically homogeneous, white, crystalline **3**, which is too unstable for satisfactory analysis. Spectral evidence, however, strongly supports the assigned structure.

The ultraviolet spectrum of the hydrazone [λ_{max} (EtOH) 233 nm (ϵ 3900)] compares favorably with that of ketone methylhydrazones [λ_{max} (EtOH) 228 nm (ϵ 4700)].[5] High-field ^1H NMR in methanol-d_4 clearly shows the presence of the terminal methyl group of the lower side chain and the methylene adjacent to the carboxylate in the upper side chain. The methylene protons adjacent to NH at C_{15} appear downfield as a doublet of triplets. The larger of the coupling constants (J = 7 Hz) is assigned to the two equivalent adjacent methylene protons at C_{16}. The smaller coupling constant (J = 2 Hz) cannot be assigned to a coupling between the methylene protons in question and the proton on nitrogen and must represent a six-bond coupling either to the methine proton at C_8 or one of the methylene protons at C_{11}. This is based on the fact that, unlike the ^1H NMR in $CDCl_3$, resonance signals for the carboxylate and nitrogen protons are not observed in CD_3OD and have presumably exchanged with methanol deuterium. In support of this argument, others have reported similar long-range coupling for other hydrazone derivatives.[5]

The hydrazone **3**, like the hydrazide **4**, can exist in two possible isomeric states about the carbon–nitrogen double bond. Again, we do not have direct evidence for a particular configuration; however, ^{13}C NMR suggests that the derivative is not a mixture of the two possible isomers. In addition, ^{13}C NMR shows only two downfield resonances for the carbonyl and hydrazone carbons, eliminating the possibility that the double bond has migrated into the five-membered ring, which would require a second olefinic resonance.

Quite unlike aromatic hydrazones and the hydrazide **4**, which both have extended conjugation and are stable, the alkyl hydrazone **3** is highly unstable, rapidly decomposing to a yellow oil with the apparent evolution of gas at room temperature. Samples of this compound that were allowed to set at room temperature overnight exhibited complete loss of the UV-absorbing spot attributed to **3** with the appearance of several new spots visualized with iodine. On the other hand, the crystalline derivative **3** appears to be stable if stored under liquid nitrogen or in dilute alcohol at room temperature. Thus, at room temperature a 6.5 mM alcohol solution of **3** shows no changes in λ_{max} or ϵ values over a period of 3 days.

Concentration of this solution, followed by TLC, further supports the conclusion that significant decomposition had not occurred under these conditions. Similar results were obtained with samples stored under liquid nitrogen over a period of weeks. As such, all pharmacological evaluations of this derivative were carried out with dilute ethanol solutions of material that had been stored under liquid nitrogen.

Pharmacology. Results with the stable hydrazide **4** indicate that it is a weaker inhibitor of arachidonic acid (AA) induced aggregation in human platelet-rich plasma (PRP) than is *trans*-13-azaprostanoic acid, and unlike *trans*-13-azaprostanoic acid,[2] its pharmacological effect is not at the TXA_2 receptor level. Thus, human platelet

(2) Le Breton, G. C.; Venton, D. L.; Enke, S. E.; Halushka, P. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 4097.
(3) Novak, L; Szantay, C. *Synthesis* **1974**, 353.
(4) Ghali, N. I.; Venton, D. L.; Hung, S. C.; Le Breton, G. C. *J. Org. Chem.* **1981**, *46*(26), 5413.
(5) Karabatsos, G. J.; Taller, R. A.; Vane, F. M. *Tetrahedron Lett.* **1964**, *18*, 1081.

Table I. Effect of Hydrazide 4 vs. *trans*-13-APA on Arachidonic Acid (AA) and U46619 Induced Human Platelet Aggregation[a]

compd	concn, μM	AA (500 μM)	U46619 (1 μM)
hydrazide 4	25	8 ± 5	
hydrazide 4	50	17 ± 4	6 ± 6
hydrazide 4	100	98 ± 2	30 ± 23
trans-13-APA	25	100 ± 8	76 ± 12
trans-13-APA	50	100 ± 0	98 ± 2

[a] PRP was incubated with 25-100 μM hydrazide 4 for 1 min prior to the addition of AA or U46619. Values represent percent inhibition of aggregation relative to vehicle controls. Percent inhibition is calculated at 3 min subsequent to addition of the agonist. *trans*-13-APA values are included for comparison purposes. The results are the mean plus or minus SEM of values obtained from at least three separate blood donors.

aggregation studies from several blood donors indicated that 100 μM **4** (1 min incubation time) was required to produce roughly 100% inhibition of aggregation induced by 500 μM AA. Lower doses showed a dose-dependent relationship of inhibition (Table I). However, even at 100 μM the hydrazide **4** produced only 30% inhibition of aggregation in response to the direct receptor agonist U46619 at 1 μM (Upjohn).[6] These results are in contrast to those obtained with 13-azaprostanoic acid, which produced 100% inhibition of aggregation induced by AA and U46619 at 25 and 50 μM, respectively.[7]

Results with the unstable hydrazone derivative **3** indicate it to be a potent and time-dependent inhibitor of AA-induced aggregation, with its site of action probably at the cyclooxygenase level. Human PRP was maximally aggregated with 500 μM AA. Pretreatment of PRP for 1 min with **3** (1 μM) repeatedly gave 99 ± 1% inhibition of the aggregation response (average of 25 donors). At lower doses (0.1 μM), complete inhibition of aggregation was not observed for 1-min incubation times, i.e., 44 ± 17% inhibition (average of nine donors). If, however, incubation times were increased, platelet aggregation was totally inhibited at 0.1 μM. In these experiments, PRP was incubated with 0.1 μM hydrazone, and samples of plasma were monitored at 5-min intervals for inhibition of aggregation. In 13 separate donors it was found that the average time for complete inhibition of aggregation (induced by 500 μM AA) was 27 ± 5 min. On the other hand, inhibition by *trans*-13-APA did not show a comparable time dependence. In each experiment, vehicle controls were also performed to establish that the observed inhibition was not due to decreased platelet responsiveness during the course of incubation. The results from these studies indicated that platelet aggregation did not decrease in response to AA (500 μM), ADP (5 μM), or U46619 (1 μM) throughout the incubation period.

In contrast, 100 μM hydrazone **3** had no effect on aggregation induced by U46619 (1–3 μM) or ADP (5 μM), i.e., 4 ± 2% inhibition and 6 ± 5% inhibition, respectively (average of nine donors). Results obtained with PGH_2 in washed platelets indicated that 1 μM hydrazone **3** did not inhibit aggregation induced by 0.6 μM PGH_2 (6 ± 5% inhibition; eight donors), although 10 μM hydrazone did produce variable inhibition and 100 μM produced almost complete inhibition (89 ± 5% inhibition; seven donors). It appears that at 1 μM hydrazone, the major site of action can be tentatively assigned to the cyclooxygenase enzyme in the AA cascade.[7] On the other hand, at higher concentrations (>10 μM), inhibition of thromboxane synthetase may be involved in the observed inhibition of platelet aggregation by this compound.

As previously mentioned, spectral data suggest that the hydrazone **3** is stable in alcohol. This stability in alcohol appears to be further documented by biological assays. Thus, a 10 mM hydrazone solution in ethanol was allowed to set at room temperature, and its effects on AA-induced aggregation were monitored. Over a period of 3 days it was shown to completely inhibit aggregation to 500 μM AA at a final concentration of 1 μM.

In order to further determine whether the hydrazone **3** or its presumptive decomposition products were responsible for the observed time-dependent inhibition of AA-induced platelet aggregation, the hydrazone was first incubated in platelet-free plasma (PFP) before addition to plasma containing platelets (PRP). At zero time, 5 μL of a 10 mM solution of hydrazone in ethanol was added to 500 μL of PFP (final concentration 100 μM) and allowed to stir. Addition of 5 μL of this mixture to a 500-μL sample of PRP (final concentration 1 μM) at 10-min intervals up to 1 h showed decreasing inhibition to aggregation with 500 μM AA as the incubation time with the PFP increased, i.e., after 60 min in PFP, 1 μM hydrazone **3** produced only 29 ± 15% inhibition of aggregation (average of seven donors). Controls in which the hydrazone (final concentration 1 μM) was added directly to the same plasma showed the expected 100% inhibition to 500 μM AA over the period of the experiment. The observed time-dependent inhibition of AA-induced platelet aggregation therefore appears to require the presence of the platelet (which contains the cyclooxygenase enzyme).

Discussion

It is tempting to try and correlate the chemical properties of *trans*-13-azaprostanoic acid (**1**), the hydrazone **3**, and the hydrazide **4** with their corresponding biological properties, although clearly this must remain largely speculative until further work is done.

Substitution of nitrogen for carbon at C_{14} and inclusion of a double bond between C_{12} and the nitrogen in the 13-azaprostanoic acid (**1**) would not seem to represent a substantial structural alteration in going from the azaprostanoid **1** to the hydrazone **3**. Apparently, however, such changes are sufficient to shift the major site of action from the presumptive TXA_2 receptor to the cyclooxygenase enzyme in the platelet.

Such a cursory structural examination does not take into account the corresponding geometrical changes, as well as the large increase in pK_b of the nitrogens in the hydrazone **3** relative to the amino acid **1**.[8] This latter difference is also seen in their relative solubilities. The derivative **1** acts like a typical aliphatic zwitterionic amino acid, relatively insoluble in water and organic solvents, whereas the hydrazone **3** acts much more like the fatty acid which it presumably mimics (AA). This latter derivative is highly soluble in organic solvents but not in water. Similar arguments may be made for the hydrazide **4**.

The instability of hydrazone **3** when compared with the azaprostanoids **4** and **1** suggests a rationale for the time-dependent inhibition of aggregation observed for this derivative. One might speculate that upon binding the hydrazone, the enzyme is irreversibly inactivated by the compound's decomposition, while the stable azaprosta-

(6) Bundy, G. L. *Tetrahedron Lett.* **1975**, *24*, 1957.
(7) For further discussion of the evaluation of the site of action of prostaglandin modulators in platelets, see ref 2.
(8) Smith, P. A. S. "The Chemistry of Open Chain Nitrogen Compounds", W. A. Benjamin: New York, 1966; Vol. 2, Chapter 9.

noids 1 and 4 act as simple reversible inhibitors.

Lands and co-workers have reported that aspirin and indomethacin, but not all nonsteroidal antiinflammatory agents, irreversibly inhibit the isolated cyclooxygenase enzyme.[9-12] Aspirin is believed to cause such inhibition by acylation of the enzyme. It is not clear what mechanism is involved with indomethacin. We have examined indomethacin's ability to inhibit AA-induced platelet aggregation and find that it also produces a time-dependent inhibition paralleling that of the hydrazone **3** but being about one order of magnitude less potent. Regardless of the detailed mechanism by which these agents are expressing their antiplatelet effects, it is clear that experimental protocol should take into consideration potential time dependence when dealing with agents that have their biochemical lesion at the cyclooxygenase level.

Experimental Section

All melting points were determined in capillary tubes on a Thomas-Hoover melting point apparatus and are uncorrected. Low-resolution NMR spectra were recorded by means of a Varian T-60A spectrometer equipped with a Nicolet TT-7 Fourier-transform accessory. High-resolution (^{13}C and ^{1}H) NMR were recorded on Brucker CXP-180 and Nicolet-150 spectrometers. Chemical shifts are reported in parts per million (ppm) relative to solvent signals in parts per million (δ): methanol, 49.0 (^{13}C), 3.30 (^{1}H); chloroform 77.0 (^{13}C), 7.24 (^{1}H); dimethyl sulfoxide, 2.49 (^{1}H). Mass spectra (MS) were obtained by Richard Dvorak at 70 eV with a Hitachi Perkin-Elmer RMU-6D single-focusing mass spectrometer and are reported as m/e (relative intensity). High-resolution mass spectra were obtained by Mr. Robins at 70 eV with a Varian MAT 112 S spectrometer. Ultraviolet spectra (UV) were recorded with a Cary 118C spectrometer and measured against the indicated solvent as reference. Microanalyses were performed by Micro-Tech Laboratories, Skokie, IL, 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 strips coated with silica gel and fluorescent indicator (Eastman Chromagram Sheet 6060). Spots were visualized by UV light and/or iodine vapor.

2-(6-Carboxyhexyl)cyclopentanone Hexylhydrazone (3). A solution of the keto acid (**2**;[3] 1.06 g, 5 mmol), *n*-hexylhydrazine hydrochloride (**10**; 0.76 g, 5 mmol), and NaOAc (0.50 g, 6.1 mmol) in MeOH (20 mL) was allowed to set overnight in the refigerator. All subsequent procedures were carried out in a cold room at 4 °C. The MeOH was removed from the cold solution with a pump attached to a roto evaporator without the aid of a water bath. The residue so obtained was dissolved in anhydrous ether (100 mL) and filtered, and the filtrate was quickly washed with ice–water (3 × 5 mL). The ether solution was dried to give a white solid (1.36 g, mp 28–33 °C), which showed only minor impurities by TLC. The product used in all experiments was recrystallized one time from ether, by the addition of petroleum ether at dry ice-2-propanol temperature, to yield the pure hydrazone (**3**; 0.92 g, 59%) as a white crystalline solid, which was stored under liquid nitrogen: mp 37–39 °C dec; TLC ($CHCl_3$–2-propanol, 20:1) R_f 0.22; UV (EtOH) λ_{max} 233 (ϵ 3900), 206 (3600), no change in λ_{max} or ϵ was observed for this solution up to 3 days; ^{1}H NMR (CD_3OD) δ 0.90 (m, 3, CH_3), 3.19 (d of t, 2, NCH_2); ^{1}H NMR ($CDCl_3$) δ 6.03 (D_2O exch, 2, NH and COOH); ^{13}C NMR (CD_3OD, 0 °C) δ 177.8 (C=O), 166.2 (C=N), 51.8, 45.5, 35.0, 33.9, 33.0, 32.1, 30.6, 30.1, 28.5, 28.3, 27.8, 26.1, 23.7, 23.6, 14.5; MS, m/e 310 (23, M^+), 239 (20), 182 (59), 111 (14), 100 (12), 84 (100). High-resolution MS M^+ Calcd ($C_{18}H_{34}N_2O_2$): 310.2622. Found: 310.2618.

2-(6-Carboxyhexyl)cyclopentylidenecaproic Acid Hydrazide (4). To a solution of the keto acid (**2**;[3] 0.60 g, 2.8 mmol) in MeOH (30 mL) was added caproic acid hydrazide (0.37 g, 2.8 mmol). The mixture was refluxed for 2 h, and the solvent was removed to give crude hydrazide (**4**; 0.90 g, mp 97–105 °C). Recrystallization (2 times) from ether gave the analytical sample (0.51 g, 56%): mp 104.5–105 °C; MS, m/e 324 (M^+); UV (EtOH) λ_{max} 234 nm (ϵ 11000), sh 215 (8400); TLC (ether) R_f 0.29; ^{1}H NMR ($CDCl_3$) 0.9 (m, 3, CH_3), 2.1–2.8 (m, 4, CH_2CO_2 and CH_2CO), 9.8 and 11.1 (br s, 1, COOH and NH); ^{13}C NMR ($CDCl_3$) δ 178, 177 (COOH, C=O), 164 (C=N), 44.7, 34.1, 32.5, 32.1, 31.7, 31.4, 29.4, 29.1, 27.6, 27.4, 24.9, 24.5, 22.8, 22.4, 13.9; MS, m/e 324 (M^+). Anl. ($C_{18}H_{32}O_3N_2$) C, H, N.

Caproic Acid Hydrazide (5). A solution of methyl hexanoate (10 g, 7.7 mmol) and 95% hydrazine (5 g, 0.15 M NH_2NH_2) in methanol (100 mL) was heated on a steam bath for 2 h. Water (10 mL) was then added, and the solvents were removed under reduced pressure to give the crude hydrazide (10 g, mp 66–70 °C). The crude product was recrystallized from chloroform to give white crystals of hydrazide **5** (8.5 g, 85%, mp 71–72 °C). The analytical sample was prepared by further recrystallization from chloroform: mp 72.5–73.5 °C (lit.[13] 76–78 °C); MS, m/e 130 (M^+); ^{1}H NMR ($CDCl_3$) δ 0.88 (m, 3, CH_3), 2.2 (m, 2, CCH_2), 4.0 (br s, 2, NH_2), 8.0 (br s, 1, NH). Anal. ($C_6H_{14}ON_2$) C, H, N.

***tert*-Butyl *n*-Hexylidinecarbazate (8).** A hexane solution containing *n*-hexanal (1.0 g, 10 mmol) and *tert*-butyl carbazate (1.3 g, 10 mmol) was heated to reflux for 20 min. While the solution cooled, *tert*-butyl *n*-hexylidenecarbazate (**8**) crystallized and was filtered (85%). Further concentration of the mother liquor separated the remainder of **8** (10%). The combined products (2.0 g, 93%) had essentially the same melting point as that of the analytical sample pepared by a single recrystallization from ether/methanol: mp 86–87 °C; MS, m/e 214 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 1.42 [s, 9, $(CH_3)_3$], 7.29 (t, 1, HC=N). Anal. ($C_{11}H_{22}N_2O_2O$) C, H, N.

***n*-Hexylhydrazine Hydrochloride (10).** To solid *tert*-butyl *n*-hexylidinecarbazate (2.0 g, 9.3 mmol) was added BH_3·THF (10 mL of a 1 M solution, 10 mmol), and the reaction mixture was allowed to stir for 10 min. HCl (6 N, 5 mL) was then added dropwise to the reaction mixture. Care must be taken at this stage, since the reaction may become vigorous with the evoltuion of isobutylene and carbon dioxide. The reaction mixture was heated for 10 min on the steam bath and then taken to dryness under reduced pressure. The residue was treated with THF (20 mL), and boric acid was removed by filtration. Removal of the solvent under reduced pressure and a single crystallization from THF/ether gave the analytical sample (1.37 g, 96%): mp 51–52 °C; MS m/e 116 (M^+ HCl salt, $C_6H_{15}N_2$), 45 (CH_2=$NHNH_2$); ^{1}H NMR ($CDCl_3$) δ 3.22 (m, 2, $NHCH_2$), 7.3 (br s, 4, $NHNH_3^+$ Cl^-). Anal. ($C_6H_{16}N_2$·HCl), C, H, N.

Stability Studies on Hydrazone 3. Hydrazone **3** (2 mg, 6.5 mmol) was dissolved in 95% ethanol (50 mL), and the UV spectrum was monitored over a period of 3 days. No change in λ_{max} (233) or ϵ (3900) was observed. After 3 days, the stock solution was concentrated under a stream of nitrogen. Examination of the residue (which still contained some ethanol) by TLC showed a single spot [R_f 0.22 ($CHCl_3$–2-propanol, 20:1)] corresponding to the starting hydrazone **3**.

Hydrazone **3** was allowed to set in an open flask overnight. Decomposition occurred as the material warmed to room temperature; i.e., the white crystals turned to a yellow oil and bubbles of gas formed in the oil. After the oil was allowed to settle overnight, TLC examination showed complete loss of the UV-absorbing spot at R_f 0.22 ($CHCl_3$–2-propanol, 20:1). A major spot at the origin and several minor spots were observed on iodine visualization. The ^{1}H NMR of this residue indicates that the C_{15} protons at δ 3.19 ($CDCl_3$) in the parent **3** are no longer present. With the exception of the peak at 84 amu in the MS, the fragmentation pattern of the residue shows none of the major fragments observed in freshly prepared **3**. Similar experiments in which **3** is rapidly heated to 40 °C indicate essentially complete decomposition.

Biological Methods. Platelet aggregation was studied by the turbidometric method of Born[14] at 37 °C over a 3-min time course.

(9) Smith, W. L.; Lands, W. E. M. *J. Biol. Chem.* **1971**, *246*, 6700.
(10) Rome, L. H.; Lands, W. E. M.; Roth, G. J.; Majerus, P. W. *Prostaglandins* **1976**, *11*, 23.
(11) Roth, C. J.; Stanford, N.; Majerus, P. W. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 3073.
(12) Rome, L. H.; Lands, W. E. M. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 4863.
(13) Volovel'skii, L. N. *Biol. Akt. Soedin., Adap. Nauk SSSR* **1965**, 202; *Chem. Abstr.* **1965**, *63*, 14935a.

Citrated human PRP from normal, healthy donors, who had denied receiving medication for 10 days, was purchased from a commercial blood bank. The plasma was centrifuged at 164g 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 PRP to give the specified concentration (see Pharmacology section).

Washed platelets were prepared by supplementing PRP with ethylenediaminetetraacetic acid (EDTA, 2.5 mM final concentration) and centrifuging the plasma at 800g for 10 min. The platelet-poor plasma was decanted, and the platelet pellet was resuspended in Ca^{2+}-free Tyrode buffer. Immediately prior to the aggregaton studies, the platelet suspension was supplemented with $CaCl_2$ (1 mM final concentration) and 0.1 mg/mL of fibrinogen.

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Registry No. 1, 71629-07-7; 2, 5288-67-5; 3, 85421-76-7; 4, 85421-77-8; 5, 2443-62-1; 6, 66-25-1; 7, 870-46-2; 8, 79201-37-9; 10, 79201-41-5; methyl hexanoate, 106-70-7; hydrazine, 302-01-2.

(14) Born, G. V. R. *Nature (London)* **1962**, *194*, 927.

Interaction of Conformationally Flexible Agonists with the Active Site of Sweet Taste. A Study of Arylureas

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The conformation of tolylureas has been studied by means of X-ray diffraction, NMR spectroscopy, and semiempirical quantum-mechanical calculations. The flat shape of meta and para isomers allows a good interaction with the model sites for bitter and sweet taste, respectively, whereas the ortho isomer cannot fit the sites because of the relative arrangements of the aryl and amide planes and because of poor hydrophobic interactions. The consistency of the conformational results with the sweet taste model site, previously proposed by the authors, is emphasized by the good fit of dulcine, a sweeter para-substituted arylurea.

Many attempts have been made in the last few years to give an interpretation of the sweet taste on the basis of the molecular structure of sweet compounds.[1-5] Besides its intrinsic importance for practical applications, this problem represents a unique opportunity in the field of quantitative structure–activity correlations, owing to the very large number of known compounds (of widely different chemical characteristics) that can impart this stimulus.[6-8] The first successful correlation among molecules as diverse as sugar, amino acids, saccharine, chloroform, nitroanilines, etc. was put forward by Shallenberger and Acree,[1] who identified an entity composed of two hydrogen-bonding groups, 2.5–3 Å apart, in all known sweet molecules. Such an entity, commonly referred as the AH–B entity, should bind to two complementary hydrogen-bonding groups located in the active site of the receptor protein. This hypothesis has been widely accepted and represents, in many cases, the only recognizable common feature among sweet tastants; however, it suffers from several notable exceptions and is certainly insufficient to explain quantitative differences in sweetening powers. To quote but one exception, it is very difficult to consider the aromatic CH group of *m*-nitroanilines[9] as a likely hydrogen bond donor.

In the last decade, several researchers have tried to identify other electronic features that, combined with the AH–B entity, could possibly account for quantitative differences in taste.

Some of these proposals refer uniquely to the possible importance of some functional groups,[4,5,10] e.g. CN, NO_2, CO_2^-, and NH_2, without any explicit reference to their spatial location with respect to the AH–B entity. Others refer to the importance of dispersion forces, often associated with the presence of aromatic rings in sweet tastants.[4,11,12]

A combination of these ideas with the need of taking into account a precise steric relationship of new subsites with respect to the AH–B entity is present in the popular model of Kier.[2] This author suggests the existence of a third subsite, hydrophobic in nature, at the apex of a triangle whose basis is formed by the AH–B entity. This third site has been identified, in turn, with an aromatic carbon atom, a double bond, etc. It is likely that all these hypotheses reflect some true feature of the active site, but they share a common weak point: all of them disregard completely two very important prerequisites, i.e., the importance of the three-dimensional shape of the agonists and the limitation imposed by the sheer (equilibrium) volume of the active site. It is trivial to note that a molecule possessing Kier's third site and an ideal AH–B entity will, notwithstanding, never elicit sweet taste if it is so large as not to enter the receptor molecule.

It is therefore necessary to identify first the main sterical features of the active site and then proceed to unravel new electronic features. An important step along this direction is represented, in our opinion, by the model we proposed a few years ago.[3] This model was built on the basis of the

(1) R. S. Shallenberger and T. E. Acree, *Nature (London)*, **216**, 480 (1967).
(2) L. B. Kier, *J. Pharm. Sci.*, **61**, 1394 (1972).
(3) (a) P. A. Temussi, F. Lelj, and T. Tancredi, *J. Med. Chem.*, **21**, 1154 (1978). (b) T. Tancredi, F. Lelj, and P. A. Temussi, *Chem. Senses Flavour*, **4**, 259 (1979).
(4) G. A. Crosby and G. E. Du Bois, *Trends Pharm. Sci.*, 372 (1980).
(5) J. M. Tinti, C. Nofre, and D. Durozard, *Naturwissenshaften*, **68**, 143 (1981).
(6) P. E. Verkade, *Farmaco, Ed. Sci.*, **23**, 248 (1968).
(7) R. A. Moncrieff, in "Chemical Senses", Leonard Hill, London, 1967, pp 507–522.
(8) R. W. Bragg, Y. Chow, L. Dennis, L. N. Ferguson, S. Howell, G. Morga, C. Ogino, H. Pugh, and M. Winters, *J. Chem. Ed.*, **55**, 281 (1978).
(9) W. Guild, *J. Chem. Ed.*, **49**, 171 (1972).
(10) J. M. Tinti, D. Durozard, and C. Nofre, *Naturwissenshaften*, **67**, 193 (1980).
(11) R. H. Mazur, A. H. Goldkamp, P. A. James, and J. M. Schlatter, *J. Med. Chem.*, **13**, 1217 (1970).
(12) M. Goodman and G. Gilon in "Peptides 1974", Y. Wolman, Ed., Halsted Press, New York, 1975, pp 271–278.

0022-2623/83/1826-1060$01.50/0