Synthesis and Platelet Aggregation-Inhibitory Activities of Novel 3-(2-Oxopropylidene)azetidin-2-one Derivatives. I[1)]

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Treatment of (*E*)-3-(2-hydroxypropylidene)-4-methyl-1-phenylazetidin-2-one (11) with 10% Pd/C gave (*E*)-(12), (*Z*)-3-(2-oxopropylidene)-4-methyl-1-phenylazetidin-2-one (13), 3,4-*cis*-(14a) and 3,4-*trans*-3-(2-oxopropyl)-4-methyl-1-phenylazetidin-2-one (14b). Among them, 12 and 13 were found to show potent inhibitory activities against rabbit platelet-rich plasma aggregation induced by adenosine diphosphate or collagen. Ring-expanded homologous derivatives and an acyclic analogue of 12 were also synthesized and tested for the biological activities. The azetidin-2-one skeleton bearing a 2-oxoalkylidene moiety at the 3 position was found to be essential for the platelet aggregation inhibitory activities of these compounds.

Keywords 3-(2-oxopropylidene)-4-methyl-1-phenylazetidin-2-one; azetidin-2-one; platelet aggregation inhibition; adenosine diphosphate; collagen; structure–activity relationship

In the previous paper in this series,[2)] we have demonstrated that 1-aryl-3-(2-hydroxyalkylidene)azetidin-2-ones are easily converted to furo[3,2-*c*]quinolines or 2-anilinomethyl-2,3-butenolides under acidic conditions (Chart 1). In the course of the further investigation of 4-substituted-1-phenyl-2-azetidinones, we have synthesized (*E*)-3-(2-hydroxypropylidene)-4-methyl-1-phenylazetidin-2-one (**11**) to examine the acid catalyzed rearrangement. Although **11** was not converted to either the corresponding 4-methylfuro[3,2-*c*]quinoline or 2-(1-anilinoethyl)-4-methyl-2,3-butenolide under acidic conditions, the novel reactivity toward 10% Pd/C was found to give the enone (**12**) and isomerized *Z* form enone (**13**) accompanied with rearranged ketone derivatives (**14a, b**).

During the biological evaluation of these monocyclic 1-arylazetidin-2-ones, **12** and **13** were found to show potent inhibitory activities against rabbit platelet-rich plasma (PRP) aggregation induced by adenosine diphosphate (ADP) or collagen *in vitro*.

Since some β-lactam antibiotics such as nafcillin or penicillin G are reported to show platelet aggregation-inhibitory activities at higher concentrations,[3)] the ring-expanded homologous compounds such as 3-(2-oxopropylidene)-1-phenylpyrrolidin-2-one (**21**) or 3-(2-oxopropylidene)-1-phenylpiperidin-2-one (**28**), and an acyclic analogue (**36**) were also synthesized to examine the structure activity relationships.

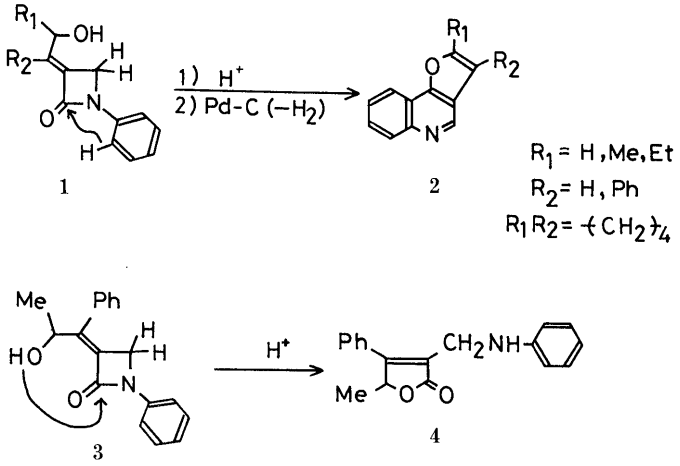


Chart 1

Furthermore, oxidation of the enol acid (**30**) obtained by alkaline hydrolysis of **11** with activated MnO_2 was examined to prepare the acyclic analogue of **12**, but the pyrrole derivative (**32**) was obtained instead of the corresponding enone. Synthesis of these compounds and the results of biological evaluations are described in this paper.

Chemistry The synthesis of **11** was effectively accomplished in a stereoselective manner according to the method reported previously, as shown in Chart 2. Lithiation of 4-methyl-1-phenylazetidin-2-one (**5**)[4)] with lithium diisopropylamide (LDA) followed by condensation with the ester (**6**)[5)] in tetrahydrofuran (THF) at −78 °C gave 3-acylazetidin-2-one derivatives (**7a, b**) as a mixture of inseparable diastereomers involving the SCH_3 group in 3:2 ratio. The stereochemistry of **7a** and **7b** was determined to be 3,4-*trans* based on the coupling constant (3 Hz) of **7a** and **7b** between C_3-H and C_4-H. Reduction of the ketone moiety of **7a, b** by $NaBH_4$ in MeOH at −78 °C proceeded in a stereoselective manner through the sodium cation-chelated intermediate[6)] to give the corresponding α alcohols (**8a, b**) in a 3:2 ratio in 89% yield, and the configuration of the hydroxy group was confirmed by the selective formation of the *E* form olefin **11** as described below. The major isomer (**8a**) was isolated by crystallization of the oily product from ether as a colorless amorphous solid in 33% yield, but the minor isomer (**8b**) obtained from the mother liquor as a viscous oil was contaminated with **8a**, and could not be further purified. Then **8a** was treated with methyl iodide in MeOH to give the sulfonium salt (**9**), which was treated successively with *tert*-BuOK at 0 °C to give **11** as a single isomer through ring cleavage of the oxirane intermediate (**10**). The geometry of the olefin moiety of **11** was determined to be *E* from the chemical shift of the olefinic proton signal (δ: 6.22) observed in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum.

Subsequently, the acid catalyzed rearrangement of **11** was examined by heating with CF_3CO_2H followed by treatment with Pd/C in toluene under reflux. Although neither the expected furo[3,2-*c*]quinoline nor the butenolide

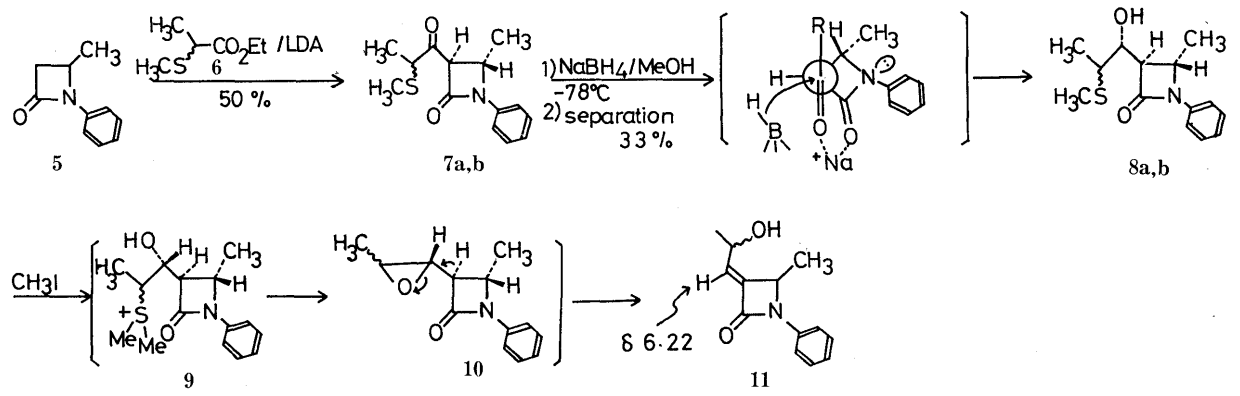


Chart 2

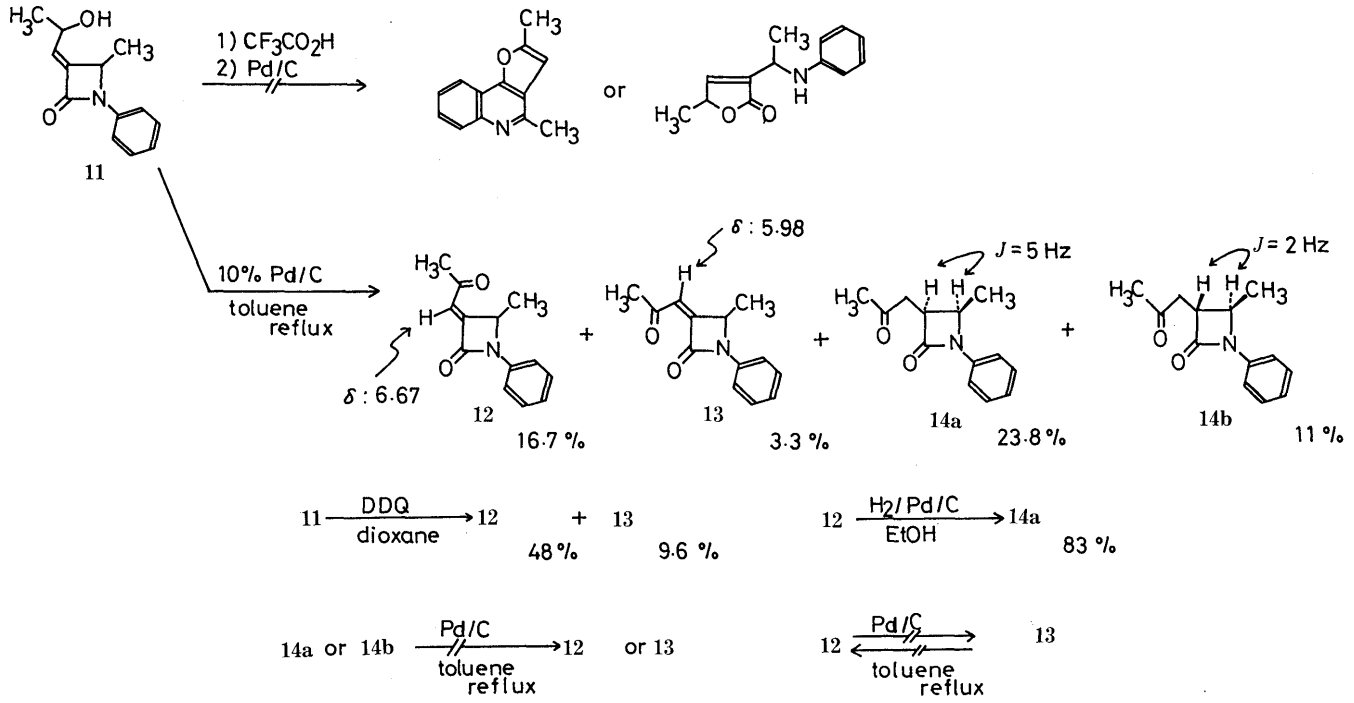


Chart 3

derivative was obtained by the acid treatment of **11**, the oxidation of the allyl alcohol moiety to a mixture of the enone derivative (**12**) and its isomer (**13**), accompanied with the formation of the rearranged ketone derivatives (**14a**, **b**) proceeded upon the treatment of **11** with Pd/C in toluene under reflux. The geometry of the enone moiety of **12** was determined to be *E* and that of **13** to be *Z* based on the characteristic olefinic proton signals observed in their ^{1}H-NMR spectra. The olefinic proton of **12** resonated at lower field (δ 6.68 ppm) than that of **13** (δ 5.98 ppm) because of the deshielding effect of the carbonyl group of the azetidin-2-one ring. For the further confirmation of the structures of **12** and **13**, alternative syntheses of **12** and **13** from **11** were investigated. Thus, the oxidation of **11** with 2,3-dichloro-5,6-dicyanoquinone (DDQ) was examined to yield **12** (48% yield) and **13** (9.6% yield). The stereochemistry of **14a** was determined as 3,4-*cis* and that of **14b** was determined as 3,4-*trans* based on the comparison of the coupling constant of **14a** (5 Hz) and **14b** (2 Hz) between C_3-H and C_4-H. The above conclusion was confirmed by the preparation of the *cis* isomer **14a** from **12** or **13** by hydrogenation on Pd/C (Chart 3).

In addition, the isomerization of **12** to **13**, and the dehydrogenation of **14a** or **14b** to **12** or **13** were not observed under the same reaction conditions as used to synthesize them from **11**.

The synthesis of ring expanded homologous derivatives of **12** was examined. Lithiation of 1-phenylpyrrolidin-2-one (**15**) with LDA followed by condensation with the ester (**16**)[7] in THF at -78 °C afforded the ketone (**17**). The ketone moiety of **17** was reduced by $NaBH_4$ in MeOH at 0 °C to give a separable mixture of isomers (**18a**; 33% yield) and (**18b**; 64% yield). The relative configuration of these isomers was determined to be as shown in Chart 5 based on the comparison of the signals of methine protons bearing a hydroxy group in their ^{1}H-NMR spectra and on the observation of intramolecular hydrogen bonding in the infrared (IR) spectrum of **18b**. The methine proton of the polar isomer (**18a**) resonated at lower field (δ 4.33 ppm) than that of the less polar isomer (**18b**) (δ 3.80 ppm) because of the deshielding effect of the carbonyl group of the pyrrolidin-2-one

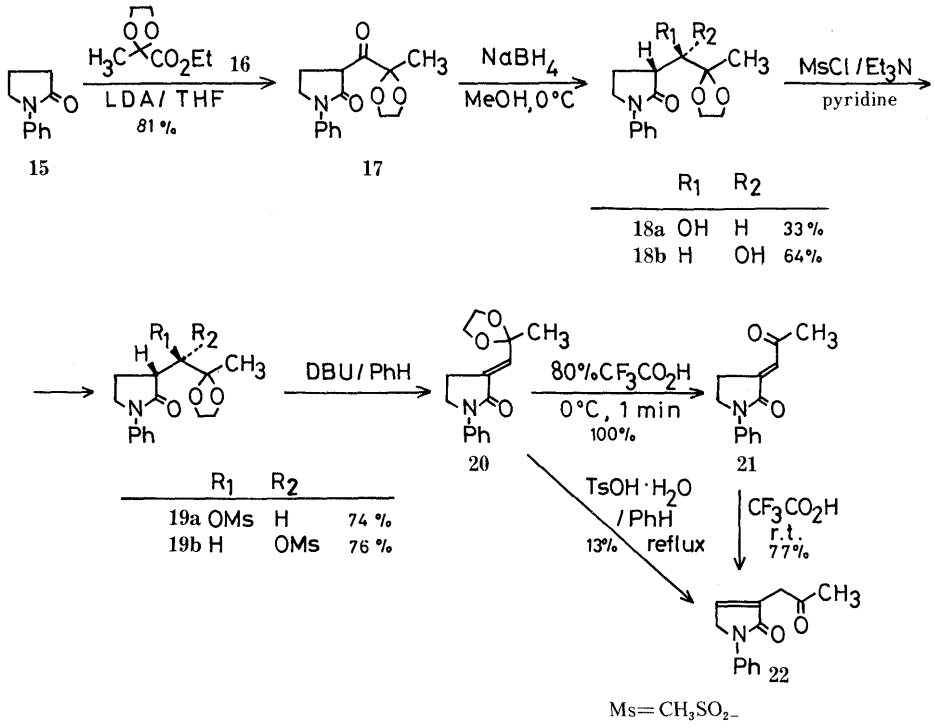
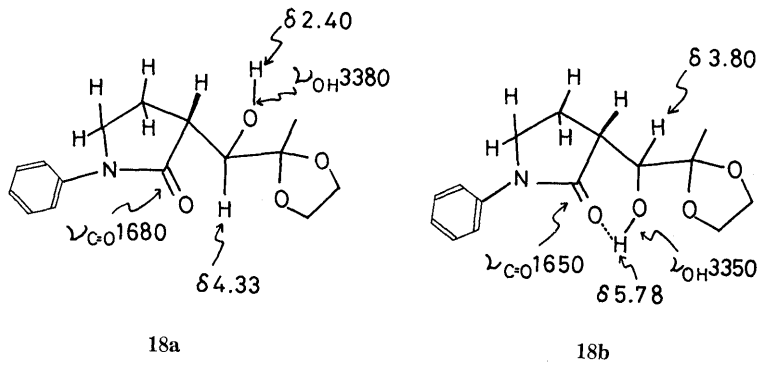


Chart 4



18a 18b

Chart 5

ring. The presence of intramolecular hydrogen bonding between the hydroxy group and carbonyl group of **18b** is indicated by the low wave number shift of the carbonyl and hydroxy absorption bands in the IR spectrum. These spectral differences between the two isomers seemed to be caused by the inhibition of free rotation of the substituent at the 3-position based on the steric hindrance between the bulky acetal group and the lactam ring. Each of **18a** and **18b** was treated with CH_3SO_2Cl to give the corresponding mesylate (**19a**) and (**19b**). Each of **19a** or **19b** was converted to 3-[(2,2-ethylenedioxy)propylidene]-1-phenylpyrrolidin-2-one (**20**) as a single isomer by treatment with excess 1.8-diazabicyclo[5.4.0]undec-7-ene (DBU) in benzene under reflux. The geometry of the enone moiety of **20** was found to be *E* based on the low field olefinic proton signals (δ 6.59) in the ^{1}H-NMR spectrum. Deprotection of the acetal of **20** to the *exo* olefin (**21**) was done by a short treatment with 80% CF_3CO_2H at 0 °C. Upon treatment with CF_3CO_2H at room temperature, **21** was isomerized to the more stable *endo* isomer (**22**) in 77% yield (Chart 4).

The piperidin-2-one homologue (**28**) was also prepared from 1-phenylpiperidin-2-one (**23**) in the same way as described for the synthesis of **21**. Reduction of the ketone (**24**) with $NaBH_4$ in MeOH gave a mixture of separable isomers (**25a**; 15% yield) and (**25b**; 37% yield). The spectral data of the polar isomer (**25a**) and the less polar isomer (**25b**) were quite similar to those of **18a** and **18b**, respectively. Mesylation of **25a** or **25b** followed by elimination with DBU gave **27** as a single product in good yield. Deprotection of **27** with 80% CF_3CO_2H at 0 °C for 0.5 min afforded the enone (**28**) in 31% yield. When this reaction was continued for 1.5 h, the *endo* isomer (**29**) was formed in 36% yield (Chart 6).

Finally, the synthesis of acyclic analogues possessing the same chromophor as **12** or **13** was examined. An attempt to cleave the azetidin-2-one ring of **12** under basic conditions failed, but the hydrolysis of **11** by aqueous KOH proceeded smoothly to give the desired amino acid derivative (**30**) in good yield. Oxidation of the allyl alcohol moiety of **30** was examined, but the enone derivative (**31**) was not isolated

and ring closure to the pyrrole derivative (**32**) proceeded even under mild conditions such as oxidation with Attemburrow's activated MnO_2[8] in ether at room temperature. The structure of **32** was confirmed by direct comparison with authentic **32** prepared by condensation of the diketone (**33**)[9] with aniline. The 3-benzoylacrylamide derivative (**36**) was synthesized from **35**[10] and aniline by *N*,*N'*-dicyclohexylcarbodiimide (DCC) condensation, but in low yield (Chart 7).

Pharmacological Results The platelet aggregation inhibitory activities of the compounds synthesized were tested on rabbit PRP *in vitro* by the method of Born,[11] and the results are summarized in Table I. 3-(2-Oxopropylidene)azetidin-2-one derivatives (**12** and **13**) both dose-dependently inhibited the first wave of ADP aggregation and collagen aggregation of rabbit PRP (Fig. 1). The IC_{50} values of **12** for ADP or collagen aggregation were calculated to be 29 or 27 μM, and those of **13** were 52 or 19 μM.

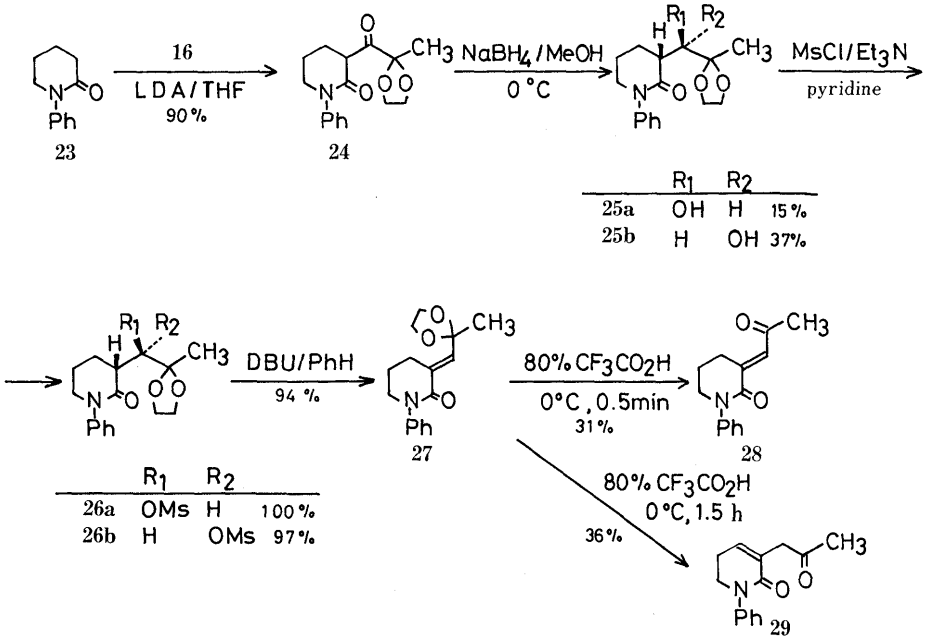
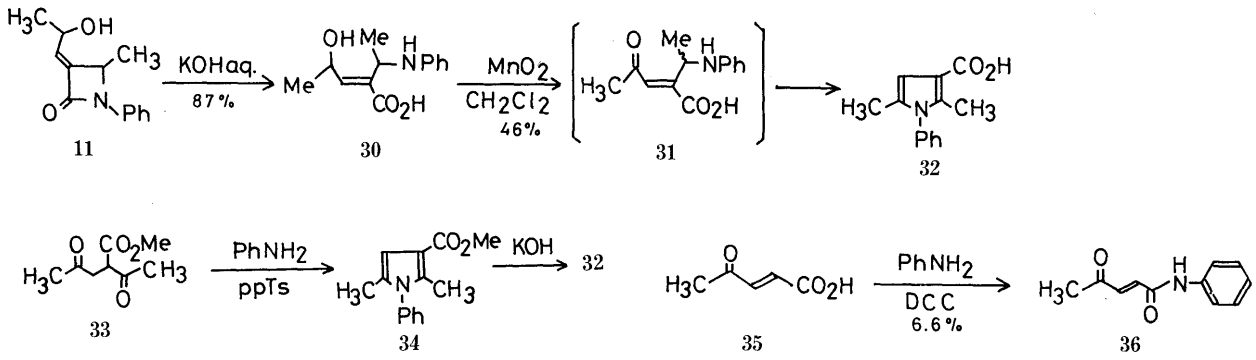


Chart 6



ppTs = pyridium *p*-toluenesulfonate

Chart 7

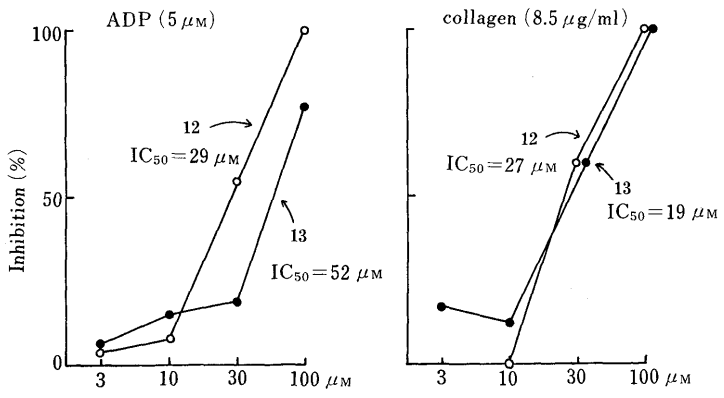


Fig. 1. Platelet Aggregation-Inhibitory Activities of **12** and **13** on Rabbit PRP

TABLE I. Platelet Aggregation Inhibitory Activities on Rabbit PRP Aggregation Induced by ADP (5 μM) or Collagen (5 μg/ml) *in Vitro*

Compound	IC_{50} (μM) ADP	Collagen
11	>100	>100
12	29.0	27.0
13	52.0	19.0
14a	>100	>100
14b	>100	>100
21	>100	>100
22	>100	>100
28	>100	>100
29	>100	>100
32	>100	>100
36	>100	>100
Aspirin	>100	35.0

However, the other compounds synthesized showed no apparent inhibitory activity at the concentration of 100 μM. From these observations, it was clear that the azetidin-2-one skeleton bearing a 2-oxoalkylidene moiety at the 3 position was essential for the platelet aggregation inhibitory activities of **12** and **13**.

Experimental

Melting points were determined on a Mettler FP-60 melting point apparatus. IR spectra were taken on a Jasco X-1A spectrometer. ^{1}H-NMR spectra were recorded with a Varian XL-200 spectrometer (Me_4Si as an internal standard, δ value), and the following abbreviations are used: singlet (s), broad singlet (br s), doublet (d), double doublet (dd), double double doublet (ddd), triplet (t), quartet (q), double quartet (dq), double triplet (dt), multiplet (m). Mass spectra (MS) were taken on a Hitachi M-80A spectrometer. Microanalytical data were obtained by using a Carlo Elba 1106R or a Perkin-Elmer 240C elemental analyzer. For column chromatography, Wakogel 200 (Wako Pure Chemical) was used, and thin layer chromatography was performed on silica gel pre-coated plates (Merck, Kieselgel 60F-254).

4-Methyl-3-(2-methylthio)propionyl-1-phenylazetidin-2-one (7) A solution of 4-methyl-1-phenylazetidin-2-one (**5**) (96.7 g, 0.60 M) in THF (505 ml) was added to a solution of LDA prepared from diisopropylamine (60.6 g, 0.60 M) and *n*-BuLi (385 ml of 1.6 N hexane solution, 0.67 M) in THF (720 ml) at −78 °C. The reaction mixture was stirred for 30 min, then a solution of ethyl 2-(methylthio)propionate (**6**) (88.8 g, 0.60 M) in THF (250 ml) was added dropwise over 1 h. The whole was stirred for 30 min at the same temperature, then water was added, and the reaction mixture was extracted with $CHCl_3$. The extract was dried (Na_2SO_4), and evaporated *in vacuo*, and the residue was purified by passing it through a short silica gel column (benzene) to give (**7a**, **b**) (78 g, yield: 50%) as a mixture of two diastereomers in a 3:2 ratio. A colorless viscous oil. IR (film): 1745 (NC=O), 1695 (C=O), 1600 (C=C) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.40 and 1.48 (3H, d, J=7 Hz, $\underline{CH_3}CHSCH_3$), 1.56 and 1.58 (3H, d, J=7 Hz, C_4-CH_3), 1.94 and 1.96 (3H, s, SCH_3), 3.50 and 3.70 (1H, q, J=7 Hz, $\underline{CH}SCH_3$), 4.20 and 4.52 (1H, d, J=3 Hz, C_3-H), 4.40 and 4.55 (1H, dq, J_q=7 Hz, J_d=3 Hz, C_4-H), 7.10 (1H, m, aromatic proton), 7.28 (4H, m, aromatic protons).

3-[1-Hydroxy-(2-methylthio)propyl]-4-methyl-1-phenylazetidin-2-one (8a, b) A solution of the mixture of **7a** and **7b** (31 g, 0.118 M) in MeOH (150 ml) was added dropwise to a stirred solution of $NaBH_4$ (8.3 g, 0.219 M) in MeOH (700 ml) at −78 °C over 2 h. The reaction mixture was stirred for 1 h at −78 °C, then AcOH (32 ml, 0.56 M) was added, and the whole was allowed to warm to room temperature. Water was added and the reaction mixture was extracted with $CHCl_3$. The extract was washed with aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated *in vacuo* to give a mixture of two diastereomers (27.8 g, 89%) in a 3:2 ratio as a viscous oil. Crystallization of the oily product from ether gave a major isomer **8a** (10.5 g, yield: 33%) as a colorless amorphous solid, mp 109.5—110.5 °C. IR (KBr): 3400 (OH), 1725 (NC=O), 1600 (C=C) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (3H, d, J=7 Hz, $\underline{CH_3}CHSCH_3$), 1.56 (3H, d, J=7 Hz, C_4-CH_3), 2.16 (3H, s, SCH_3), 2.84 (1H, d, J=5 Hz, OH), 2.90 (1H, q, J=7 Hz, $\underline{CH}SCH_3$), 3.15 (1H, dd, J=5, 3 Hz, C_3-H), 3.98 (1H, dt, J_t=5 Hz, J_d=7 Hz, $\underline{CH}OH$), 4.29 (1H, dt, J_t=7 Hz, J_d=3 Hz, C_4-H), 7.10 (1H, m, aromatic proton), 7.3—7.5 (4H, m, aromatic protons). EIMS *m/z*: 265 (M^+). *Anal.* Calcd for $C_{14}H_{19}NSO_2$: C, 63.36; H, 7.22; N, 5.28. Found: C, 63.28; H, 7.26; N, 5.27. The mother liquor yielded a minor isomer **8b** contaminated with the major isomer as a viscous oil. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (3H, d, J=7 Hz, $\underline{CH_3}CHSCH_3$), 1.55 (3H, d, J=7 Hz, C_4-H), 2.11 (3H, s, SCH_3), 3.15 (2H, m, C_3-H), 3.24 (1H, br s, OH), 3.85 (1H, dd, J=8, 5 Hz, $\underline{CH}OH$), 4.25 (1H, dt, J_t=7 Hz, J_d=3 Hz, C_4-H), 7.10 (1H, m, aromatic proton), 7.3—7.5 (4H, m, aromatic protons).

(*E*)-3-(2-Hydroxypropylidene)-4-methyl-1-phenylazetidin-2-one (11) MeI (4.82 g, 0.034 M) was added to a solution of **8a** (9 g, 0.034 M) in MeOH (330 ml), and the mixture was heated under reflux for 6 h. After removal of the solvent *in vacuo*, the resulting sulfonium salt (**9**) was dissolved in absolute EtOH (120 ml), and *tert*-BuOK (7.6 g, 67.7 mM) was added in small portions at 0 °C. The reaction mixture was stirred at room temperature for 1 h, then evaporated *in vacuo*, and the resulting residue was extracted with $CHCl_3$. The extract was washed with water, aqueous $NaHCO_3$ and brine successively, dried ($MgSO_4$) and evaporated *in vacuo* to give **11** (4.6 g, yield: 62%), which was recrystallized from ether to give a colorless amorphous solid, mp 89.5—91.5 °C. IR (KBr): 3440 (OH), 1725 (N=CO), 1590 (C=C) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, d, J=7Hz, $\underline{CH_3}CHOH$), 1.64 (3H, d, J=7 Hz, C_4-CH_3), 2.28 (1H, br s, OH), 4.60 (1H, m, $\underline{CH}OH$), 4.75 (1H, q, J=7 Hz, C_4-H), 6.22 (1H, d, J=5 Hz, HC=C), 7.10 (1H, m, aromatic proton), 7.38 (4H, m, aromatic protons). EIMS *m/z*: 217 (M^+). *Anal.* Calcd for $C_{13}H_{15}NO_2$: C, 71.87; H, 6.96; N, 6.44. Found: C, 71.88; H, 6.90; N, 6.38.

The Reaction of 11 with 10% Pd/C in Toluene under Reflux A mixture of **11** (6.1 g, 0.028 M), 10% Pd/C (3 g), and toluene (120 ml) was heated under reflux for 3 h. After removal of the Pd/C by filtration, the filtrate was evaporated *in vacuo* and the oily residue was submitted to silica gel column chromatography (CH_2Cl_2) to give **12** (1.0 g, yield: 16.7%), **13** (0.2 g, yield: 3.3%), **14a** (1.45 g, yield: 23.8%) and **14b** (0.95 g, yield: 11%). **12**: Yellow prisms from EtOH, mp 120—122 °C. IR (KBr): 1745 (NC=O), 1650 (C=CC=O), 1590 (C=C) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.63 (3H, d, J=7 Hz, C_4-CH_3), 2.37 (3H, s, CH_3C=O), 4.98 (1H, dq, J_d=2, J_q=7 Hz, C_4-H), 6.67 (1H, d, J=2 Hz, HC=C), 7.15 (1H, m, aromatic proton), 7.42 (4H, m, aromatic protons). EIMS *m/z*: 215 (M^+). *Anal.* Calcd for $C_{13}H_{13}NO_2$: C, 72.54; H, 6.09; N, 6.50. Found: C, 72.54, H, 6.10; N, 6.49. **13**: Pale yellow needles from ether, mp 114—115.5 °C. IR (KBr): 1740 (C=O), 1660 (C=CC=O), 1590 (C=C) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.61 (3H, d, J=7 Hz, C_4-CH_3), 2.74 (3H, s, CH_3C=O), 4.68 (1H, q, J=7 Hz, C_4-H), 5.98 (1H, s, HC=C), 7.20 (1H, m, aromatic proton), 7.45 (4H, m, aromatic protons). EIMS *m/z*: 215 (M^+). *Anal.* Calcd for $C_{13}H_{13}NO_2$: C, 72.54; H, 6.09; N, 6.50. Found: C, 72.61; H, 6.14; N, 6.44. **14a**: Colorless needles from EtOH, mp 114.5—116.5 °C. IR (KBr): 1745 (NC=O), 1705 (C=O), 1590 (C=C) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, d, J=7 Hz, C_4-CH_3), 2.24 (3H, s, CH_3C=O), 2.84 (1H, dd, J=20, 12 Hz, HCC=O), 3.04 (1H, dd, J=20, 5 Hz, HCC=O), 3.82 (1H, dt, J_t=5 Hz, J_d=12 Hz, C_3-H), 4.40 (1H, dq, J_q=7 Hz, J_d=5 Hz, C_4-H), 7.10 (1H, m, aromatic protons), 7.38 (4H, m, aromatic protons). EIMS *m/z*: 217 (M^+). *Anal.* Calcd for $C_{13}H_{15}NO_2$: C, 71.87; H, 6.96; N, 6.44. Found: C, 71.88; H, 6.98; N, 6.55. **14b**: A colorless viscous oil, IR (film): 1745 (NC=O), 1705 (C=O), 1590 (C=C) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.62 (3H, d, J=6 Hz, C_4-CH_3), 2.21 (3H, s, CH_3C=O), 2.70 (1H, dd, J=18, 12 Hz, HCC=O), 2.95 (1H, ddd, J=12, 3.5, 2 Hz, C_3-H), 3.13 (1H, dd, J=18, 3.5 Hz, HCC=O), 3.79 (1H, dq, J_q=6 Hz, J_d=2 Hz, C_4-H), 7.10 (1H, m, aromatic proton), 7.35 (4H, m, aromatic protons). EIMS *m/z*: 217 (M^+).

Oxidation of 11 with DDQ A mixture of **11** (20.3 mg, 0.093 mM), DDQ (28 mg, 0.12 mM), and dioxane (0.35 ml) was stirred at room temperature for 20 h. The reaction mixture was evaporated *in vacuo* and separated by silica gel column chromatography (CH_2Cl_2) to give **12** (16.2 mg, yield: 48%) and **13** (3.2 mg, yield: 9.6%).

Hydrogenation of 12 A mixture of **12** (50 mg, 0.23 mM), 10% Pd/C (30 mg), and EtOH (50 ml) was stirred at room temperature in a hydrogen atmosphere under normal pressure to give **14a** (42 mg, yield: 83%).

3-[2,2-(Ethylenedioxy)propionyl]-1-phenylpyrrolidin-2-one (17) A solution of 1-phenylpyrrolidin-2-one (**15**) (10 g, 62 mM) in THF (10 ml) was added dropwise to a stirred solution of LDA, prepared from diisopropylamine (8.94 ml, 62 mM) and *n*-BuLi (38.3 ml of 1.6 N hexane solution, 62 mM) in THF (90 ml) at −78 °C. The reaction mixture was stirred for 10 min, then a solution of ethyl 2,2-ethylenedioxypropionate (**16**) (9.9 g, 62 mM) in THF (10 ml) was added. The whole was stirred for 30 min and then poured into water, and extracted with $CHCl_3$. The extract was washed with brine, dried ($MgSO_4$) and evaporated *in vacuo* to give **17** (13.8 g, yield: 81%), which was recrystallized from $CHCl_3$–ether to give

colorless prisms, mp 84—86 °C. IR (KBr): 1730 (C=O), 1680 (NC=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.58 (3H, s, CH_3), 2.38 (2H, m, C_4-H), 3.92 (2H, m, C_5-H), 4.10 (4H, m, OCH_2CH_2O), 4.37 (1H, dd, J=8, 9 Hz, C_3-H), 7.18 (1H, m, aromatic proton), 7.39 (2H, m, aromatic protons), 7.60 (2H, m, aromatic protons). EIMS m/z: 275 (M^+). *Anal.* Calcd for $C_{15}H_{17}NO$: C, 65.44; H, 6.22; N, 5.09. Found: C, 65.42; H, 6.22; N, 5.06.

(*R)-3-[(*R**)-2,2-(Ethylenedioxy)-1-hydroxypropyl]-1-phenylpyrrolidin-2-one (18a) and (*R**)-3-[(*S**)-2,2-(Ethylenedioxy)-1-hydroxypropyl)]-1-phenylpyrrolidin-2-one (18b)** A solution of **17** (11 g, 40 mM) in MeOH (50 ml) was added dropwise to a solution of $NaBH_4$ (1.8 g, 48 mM) in MeOH (100 ml) at 0 °C. The mixture was stirred at 0 °C for 1 h, then AcOH (8 ml) was added and the whole was poured into water and extracted with CH_2Cl_2. The extract was washed with water and brine, dried ($MgSO_4$), and evaporated *in vacuo* and the residue was separated by silica gel column chromatography (CH_2Cl_2) to give **18a** (3.65 g, yield: 33%) and **18b** (7.1 g, yield: 64%). **18a**: Colorless prisms from $CHCl_3$–ether, mp 129—130 °C. IR (KBr): 3380 (OH), 1670 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, s, CH_3), 2.18 and 2.50 (each 1H, m, C_4-H), 2.40 (1H, d, J=4 Hz, OH), 2.85 (1H, br t, J=9.5 Hz, C_3-H), 3.81 (2H, dd, J=9.5, 5 Hz, C_5-H), 4.04 (4H, m, OCH_2CH_2O), 4.33 (1H, dd, J=4, 2 Hz, HOCH), 7.15 (1H, m, aromatic proton), 7.38 (2H, m, aromatic protons), 7.69 (2H, m, aromatic protons). EIMS m/z: 277 (M^+). *Anal.* Calcd for $C_{15}H_{19}NO_4$: C, 64.97; H, 6.91; N, 5.05. Found: C, 64.96; H, 6.91; N, 5.05. **18b**: Colorless prisms from $CHCl_3$–ether, mp 94—95 °C. IR (KBr): 3350 (OH), 1650 (NC=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (3H, s, CH_3), 2.12 and 2.30 (each 1H, m, C_4-H), 2.78 (1H, dt, J_d=13 Hz, J_t=8 Hz, C_3-H), 3.80 (1H, dt, J_d=2.5 Hz, J_t=10 Hz, HOCH), 3.85 (2H, m, C_5-H), 4.03 (4H, m, OCH_2CH_2O), 5.78 (1H, d, J=2 Hz, OH), 7.20 (1H, m, aromatic proton), 7.40 (2H, m, aromatic protons), 7.61 (2H, m, aromatic protons). EIMS m/z: 277 (M^+). *Anal.* Calcd for $C_{15}H_{19}NO_4$: C, 64.97; H, 6.91; N, 5.05. Found: C, 64.95; H, 6.94; N, 5.06.

(*R)-3-[(*R**)-2,2-(Ethylenedioxy)-1-methylsulfonylpropyl]-1-phenylpyrrolidin-2-one (19a)** A solution of CH_3SO_2Cl (1.1 ml, 14 mM) in CH_2Cl_2 (5 ml) was added dropwise to a solution of **18a** (3.3 g, 12 mM) and Et_3N (3.3 ml, 24 mM) in CH_2Cl_2 (50 ml) at 0 °C. The reaction mixture was stirred at room temperature for 1 h, then poured into water, washed with aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2 : ether = 10 : 1) to give **19a** (3.1 g, yield: 74%) as a colorless oil. IR (film): 1685 (NC=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.50 (3H, s, C-CH_3), 2.40 (2H, m, C_4-H), 3.10 (1H, m, C_3-H), 3.18 (3H, s, SO_2CH_3), 3.80 (2H, m, C_5-H), 4.05 (4H, m, OCH_2CH_2O), 4.93 (1H, d, J=5 Hz, HCOSO$_2$CH$_3$), 7.18 (1H, m, aromatic proton), 7.38 (2H, m, aromatic protons), 7.60 (2H, m, aromatic protons). EIMS m/z: 355 (M^+).

(*R)-3-[(*S**)-2,2-(Ethylenedioxy)-1-methylsulfonylpropyl]-1-phenylpyrrolidin-2-one (19b)** Prepared from **18b** in the same manner as used for **19a**. Colorless prisms from ether, mp 105—107 °C. IR (KBr): 1690 (NC=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (3H, s, CCH_3), 2.40 (2H, m, C_4-H), 3.03 (3H, s, SO_2CH_3), 3.12 (1H, m, C_3-H), 3.85 (2H, dd, J=10, 7 Hz, C_5-H), 4.10 (4H, m, OCH_2CH_2O), 5.25 (1H, d, J=2 Hz, HCOSO$_2$CH$_3$), 7.18 (1H, m, aromatic proton), 7.38 (2H, m, aromatic protons), 7.65 (2H, m, aromatic protons). EIMS m/z: 355 (M^+).

(*E*)-3-[(2,2-Ethylenedioxy)propylidene]-1-phenylpyrrolidin-2-one (20) A mixture of **19a** (2.5 g, 7 mM), DBU (3.2 g, 21 mM), and benzene (50 ml) was heated under reflux for 2 h, washed twice with water, dried ($MgSO_4$), and evaporated *in vacuo* to give **20** (1.5 g, 82%), which was recrystallized from hexane–$CHCl_3$ to give colorless prisms, mp 110—112 °C. IR (KBr): 1685 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.56 (3H, s, CH_3), 3.05 (2H, dt, J_d=2 Hz, J_t=7 Hz, C_4-H), 3.90 (2H, t, J=7 Hz, C_5-H), 4.00 (4H, m, OCH_2CH_2O), 6.59 (1H, t, J=2 Hz, C=CH), 7.19 (1H, m, aromatic proton), 7.41 (2H, m, aromatic protons), 7.75 (2H, m, aromatic protons). EIMS m/z: 324 (M^+). *Anal.* Calcd for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.60; N, 5.39. Found: C, 69.44; H, 6.60; N, 5.38.

(*E*)-3-(2-Oxopropylidene)-1-phenylpyrrolidin-2-one (21) Compound **20** (1.5 g, 4.67 mM) was added as a single portion to 80% CF_3CO_2H at 0 °C. After being stirred for 1 min, the reaction mixture was poured into 5% Na_2CO_3 solution and extracted with CH_2Cl_2. The extract was washed with water and brine, dried ($MgSO_4$), and evaporated *in vacuo* to give **21** (1.2 g, yield: 100%), which was recrystallized from CH_2Cl_2–ether to give pale yellow prisms, mp 152—153 °C. IR (KBr): 1660 (NC=O), 1630 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.37 (3H, s, CH_3), 3.32 (2H, dt, J_t=7 Hz, J_d=3 Hz, C_4-H), 3.96 (2H, t, J=7 Hz, C_5-H), 7.08 (1H, t, J=3 Hz, C=CH), 7.23 (1H, m, aromatic proton), 7.43 (2H, m, aromatic protons), 7.79 (2H, m, aromatic protons). EIMS m/z: 215 (M^+). *Anal.* Calcd for $C_{13}H_{13}NO_2$: C, 72.54; H, 6.09; N, 6.50. Found: C, 72.68; H, 6.32; N, 6.55.

3-(2-Oxopropyl)-2-oxo-1-phenyl-3-pyrroline (22) A mixture of **21** (560 mg, 2.6 mM) and 80% CF_3CO_2H (5 ml) was stirred at room temperature for 2 h, diluted with CH_2Cl_2, washed with aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2) to give **22** (430 mg, yield: 77%), which was recrystallized from CH_2Cl_2–iso-Pr_2O to give yellow needles, mp 95—96 °C. IR (KBr): 1710 (C=O), 1660 (NC=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.30 (3H, s, CH_3), 3.52 (2H, d, J=2 Hz, CH_2C=O), 4.23 (2H, m, C_5-H), 7.10 (1H, m, C=CH), 7.16 (1H, m, aromatic proton), 7.40 (2H, m, aromatic protons), 7.86 (2H, m, aromatic protons). EIMS m/z: 215 (M^+). *Anal.* Calcd for $C_{13}H_{13}NO_3$: C, 72.54; H, 6.09; N, 6.50. Found: C, 72.26; H, 6.08; N, 6.35.

Compounds **24** to **29** were prepared in the same manner as described above.

3-(2,2-Ethylenedioxypropionyl)-1-phenylpiperidin-2-one (**24**): A colorless viscous oil. IR (KBr): 1730 (C=O), 1635 (NC=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.53 (3H, s, CH_3), 1.95 (2H, m, C_4-H), 2.15 (2H, m, C_5-H), 3.65 (2H, m, C_6-H), 4.00 (4H, m, OCH_2CH_2O), 4.20 (1H, m, C_3-H), 7.20—7.50 (5H, m, aromatic protons). EIMS m/z: 289 (M^+).

(*R**)-3-[(*R**)-2,2-(Ethylenedioxy)-1-hydroxypropyl)]-1-phenylpiperidin-2-one (**25a**): Colorless prisms from CH_2Cl_2, mp 69—70 °C. IR (KBr): 3400 (OH), 1625 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, s, CH_3), 2.10 (4H, m, C_4-H and C_5-H), 2.80 (1H, dt, J_d=3 Hz, J_t=9 Hz, C_3-H), 2.96 (1H, d, J=7 Hz, OH), 3.65 (2H, m, C_4-H), 4.04 (4H, m, OCH_2CH_2O), 4.50 (1H, dd, J=7, 3 Hz, HCOH), 7.20—7.50 (5H, m, aromatic protons). EI MS m/z: 291 (M^+). *Anal.* Calcd for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.26; N, 4.80. Found: C, 65.76; H, 7.13; N, 4.93.

(*R**)-3-[(*S**)-2,2-(Ethylenedioxy)-1-hydroxypropyl)]-1-phenylpiperidin-2-one (**25b**): A colorless viscous oil. IR (film): 3320 (OH), 1620 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (3H, s, CH_3), 1.80—2.10 (4H, m, C_4-H and C_5-H), 2.70 (1H, m, C_3-H), 3.65 (2H, m, C_6-H), 3.82 (1H, dd, J=8, 5 Hz, HCOH), 4.00 (4H, m, OCH_2CH_2O), 5.43 (1H, d, J=5 Hz, OH), 7.20—7.50 (5H, m, aromatic protons). EIMS m/z: 291 (M^+).

(*R**)-3-[(*R**)-2,2-(Ethylenedioxy)-1-methylsulfonyloxy)]-1-phenylpiperidin-2-one (**26a**): A colorless viscous oil. IR (film): 1680 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.40 (3H, s, C-CH_3), 2.10 (4H, m, C_4-H and C_5-H), 2.95 (1H, m, C_3-H), 3.22 (3H, s, SO_2CH_3), 3.65 (2H, m, C_6-H), 4.05 (4H, m, OCH_2CH_2O), 4.95 (1H, d, J=5 Hz, HCOSO$_2$CH$_3$), 7.2—7.5 (5H, m, aromatic protons). EIMS m/z: 369 (M^+).

(*R**)-3-[(*S**)-2,2-(Ethylenedioxy)-1-methylsulfonyloxy)]-1-phenylpiperidinone (**26b**): A colorless viscous oil. IR (film): 1680 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.41 (3H, s, C-CH_3), 2.10 (4H, m, C_4-H and C_5-H), 3.01 (1H, m, C_3-H), 3.05 (3H, s, SO_2CH_3), 3.65 (2H, m, C_6-H), 4.07 (4H, m, OCH_2CH_2O), 5.33 (1H, d, J=2 Hz, HOSO$_2$CH$_3$), 7.2—7.5 (5H, m, aromatic protons). EIMS m/z: 369 (M^+).

(*E*)-3-(2,2-Ethylenedioxypropylidene)-1-phenylpiperidin-2-one (**27**): Colorless prisms from iso-Pr_2O–CH_2Cl_2, mp 118—119 °C. IR (KBr): 1655 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.57 (3H, s, CH_3), 2.02 (2H, m, C_5-H), 2.92 (2H, m, C_4-H), 3.72 (2H, m, C_6-H), 3.90 and 3.98 (each 2H, m, OCH_2CH_2O), 6.90 (1H, t, J=1 Hz, C=CH), 7.28 (3H, m, aromatic protons), 7.40 (2H, m, aromatic protons). EIMS m/z: 273 (M^+). *Anal.* Calcd for $C_{16}H_{19}NO_3$: C, 70.31; H, 7.01; N, 5.12. Found: C, 70.33; H, 7.01; N, 5.16.

(*E*)-3-(2-Oxopropylidene)-1-phenylpiperidin-2-one (**28**): Pale yellow prisms from CH_2Cl_2–ether, mp 96—98 °C. IR (KBr): 1675 (C=O), 1635 (NC=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.04 (2H, m, C_5-H), 2.34 (3H, s, CH_3), 3.17 (2H, dt, J_d=2 Hz, J_t=6 Hz, C_4-H), 3.78 (2H, t, J=6 Hz, C_6-H), 7.30 (3H, m, aromatic protons), 7.39 (1H, d, J=3 Hz, C=CH), 7.43 (2H, m, aromatic protons). EIMS m/z: 229 (M^+). *Anal.* Calcd for $C_{14}H_{15}NO_2$: C, 73.34; H, 6.59; N, 6.11. Found: C, 72.91; H, 6.67; N, 5.82.

5,6-Dihydro-2-oxo-3-(2-oxopropyl)-1-phenyl-3-piperine (**29**): Colorless prisms from CH_2Cl_2–ether, mp 87—88 °C. IR (KBr): 1685 (C=O), 1655 (NC=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.10 (2H, m, C_5-H), 2.36 (3H, s, CH_3), 2.72 (2H, dt, J_d=2 Hz, J_t=6 Hz, C_4-H), 3.76 (2H, t, J=6 Hz, C_6-H), 6.15 (1H, t, J=2 Hz, C=CH), 7.25—7.35 (3H, m, aromatic protons), 7.40 (2H, m, aromatic protons). CIMS m/z: 230 (M+H). *Anal.* Calcd for $C_{14}H_{15}NO_2$: C, 73.34; H, 6.59; N, 6.11. Found: C, 72.99; H, 6.58; N, 5.84.

(*E*)-4-Hydroxy-2-(1-phenylaminoethyl)pent-2-enoic Acid (30) A solution of KOH (1.1 g, 16 mM) in water (9 ml) was added to a solution of **11** (0.5 g, 2.3 mM) in dioxane (1 ml) at room temperature. After being stirred for 16 h, the reaction mixture was neutralized by addition of 1 N HCl and extracted twice with EtOAc. The extract was washed with brine, dried ($MgSO_4$), and evaporated *in vacuo*, and the residue was purified by silica gel column chromatography (CH_2Cl_2 : MeOH : AcOH = 200 : 20 : 1) to give

30 (470 mg, yield: 87%) as a viscous oil. IR (film): 3600 (COOH), 1700 (C=O), 1600 (C=C) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, d, J=6.5 Hz, $\underline{CH_3}CHOH$), 1.45 (3H, d, J=7 Hz, $\underline{CH_3}CHNHPh$), 4.75 (1H, q, J=7 Hz, $\underline{CH}NHPh$), 4.93 (1H, m, $\underline{CH}OH$), 6.04 (3H, br s, COOH, OH, NH), 6.55—6.75 (3H, m, aromatic protons), 6.80 (1H, d, J=8.5 Hz, HC=C), 7.05—7.2 (2H, m, aromatic protons). CIMS m/z: 236 (M+H).

2,6-Dimethyl-1-phenylpyrrole-3-carboxylic Acid (32) Method 1: Attemburrow's MnO_2 (1.74 g, 20 mM) was added to a stirred solution of **30** (220 mg, 1 mM) in ether (60 ml). The reaction mixture was stirred for 20 min at room temperature, filtered through Celite, decolorized with charcoal, and evaporated *in vacuo* to give **32** (100 mg, yield: 46%), which was recrystallized from iso-Pr_2O to give colorless prisms, mp 207—208 °C. IR (KBr): 3000 (OH), 1640 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.90 and 2.20 (each 3H, s, CH_3 × 2), 3.26 (1H, br s, COOH), 6.02 (1H, s, C_4-H), 7.0—7.65 (5H, m, aromatic protons). EIMS m/z: 215 (M^+). *Anal.* Calcd for $C_{13}H_{13}NO_2$: C, 72.54; H, 6.09; N, 6.50. Found: C, 72.47; H, 6.08; N, 6.39.

Method 2: A stirred solution of **38** (0.9 g, 4 mM) in MeOH (10 ml) was treated with 1 N NaOH (1 ml). The mixture was stirred for 3 h at room temperature, then 1 N HCl (3 ml) was added, and the reaction mixture was extracted twice with EtOAc. The extract was washed with brine, dried ($MgSO_4$), and evaporated *in vacuo* to give **32** (0.5 g, yield: 60%).

Methyl 2,6-Dimethyl-1-phenylpyrrole-3-carboxylate (34) A mixture of **33** (1.72 g, 10 mM), aniline (0.93 g, 10 mM) and pyridinium *p*-toluenesulfonate (125 mg, 0.5 mM) in benzene (50 ml) was refluxed for 1 h with removal of the resulting water under azeotropic conditions. The reaction mixture was evaporated *in vacuo* and the residue was purified by silica gel column chromatography (CH_2Cl_2) to give **34** (0.96 g, yield: 42%), which was recrystallized from hexane to give colorless needles, mp 51—54 °C. IR (KBr): 1710 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.97 (3H, s, C_5-H), 2.27 (3H, s, C_2-H), 3.73 (3H, s, CO_2CH_3), 6.23 (1H, s, C_4-H), 7.0—7.5 (5H, m, aromatic protons). EIMS m/z: 229 (M^+). *Anal.* Calcd for $C_{14}H_{15}NO_2$: C, 73.34; H, 6.59; N, 6.11. Found: C, 73.26; H,6.61; N, 6.09.

***(E)*-*N*-Phenyl 4-Oxopent-2-enamide (36)** DCC (1.5 g, 8.4 mM) in CH_2Cl_2 (50 ml) was added to a mixture of (*E*)-4-oxo-2-pentenoic acid (**35**) (1.14 g, 8.4 mM) and aniline (0.93 g, 10 mM) in CH_2Cl_2 (50 ml) at 0 °C. The mixture was stirred for 1 h at the same temperature, and the resulting precipitate was filtered off. The filtrate was washed with 1 N HCl, aqueous $NaHCO_3$ and brine successively, and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2 : ether = 10 : 1) to give **36** (120 mg, 6.6%), which was recrystallized from ether to give a yellow amorphous solid, mp 138—140 °C. IR (KBr): 3300 (NH), 1655 (C=O), 1620 (NC=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.38 (3H, s, CH_3), 6.97 (1H, d, J=15 Hz, C_3-H), 7.18 (1H, m, aromatic proton), 7.17 (2H, m, aromatic protons), 7.22 (1H, d, J=15 Hz, C_2-H), 7.64 (2H, m, aromatic protons), 8.20 (1H, br s, NH). EIMS m/z: 319 (M^+). *Anal.* Calcd for $C_{11}H_{11}NO_2$: C, 69.83; H, 5.86; N, 7.40. Found: C, 69.91; H, 5.88; N, 7.52.

Preparation of PRP Blood was taken from the carotid artery of a male New Zealand White rabbit into a plastic syringe containing 10% volume of 3.2% sodium citrate dihydrate solution under ether anesthesia. The citrated blood was centrifuged at 150 ***g*** for 15 min at room temperature to obtain PRP. The sediment was further centrifuged at 1500 ***g*** for 10 min to obtain platelet-poor plasma (PPP). The platelet count was adjusted to approximately 5×10^5—6×10^5/μl by adding PPP.

Platelet Aggregation Test *in Vitro* Platelet aggregation was measured by a Aggrecoder PA-3210 (Kyoto Daiichi Kagaku) at 37 °C under stirring at 1000 rpm. The agents used were ADP (Shigma Chemical Co., final concentration, 5 μM), and collagen (Kyoto Daiichi Kagaku Co., Ltd.; final concentration, 5 μg/ml). The compound to be tested was dissolved in DMSO, diluted with saline, and added in a volume of 25 μl to 275 μl of PRP 3 min before addition of the aggregating agents. Platelet aggregation was measured for 5 min and the IC_{50} value was calculated from the maximum decrease in absorbancy of PRP in comparison with that of vehicle-treated PRP.

References and Notes

1) A part of this work was presented at the 109th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1989.
2) S. Kano, S. Shibuya and T. Ebata, *Heterocycles*, **15**, 1011 (1981); S. Kano, T. Ebata and S. Shibuya, *J. Chem. Soc., Perkin Trans. 1*, **1980**, 2105; S. Kano, T. Ebata, Y. Yuasa and S. Shibata, *Heterocycles*, **14**, 589 (1980).
3) G. D. Qureshi, C. A. Fletcher, S. C. Choi, and R. J. Duma, *Clin. Res.*, **31**, 849A (1983); K. H. Jakobs, G. Schultz, B. Gauglar, and T. Pfeuffer, *Eur. J. Biochem.*, **134**, 351 (1983).
4) T. Kano, S. Shibuya and T. Ebata, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 257.
5) K. Tanaka, N. Yamagishi, R. Tanikaga and A. Kaji, *Bull. Chem. Soc. Jpn.*, **52**, 3619 (1979).
6) F. A. Bouffard and B. G. Christensen, *J. Org. Chem.*, **46**, 2208 (1981); R. S. Glass, D. R. Deardorff and K. Hengar, *Tetrahedron Lett.*, **21**, 2467 (1980).
7) A. Holland, J. M. Inglis and R. Slack, Brit. Patent 951115 (1964) [*Chem. Abstr.*, **60**, 15874*h* (1964)].
8) J. Attemburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jansen and T. Walker, *J. Chem. Soc.*, **1952**, 1094.
9) C. D. Hurd and K. Wilkinson, *J. Am. Chem. Soc.*, **70**, 739 (1948).
10) R. Scheffold and P. Dubs, *Helv. Chim. Acta*, **50**, 798 (1967).
11) G. V. R. Born, *Nature* (London), **194**, 927 (1962); G. V. R. Born and M. Cross, *J. Physiol.* (London), **168**, 178 (1963).