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Synthesis of Hydrazone Derivatives of 3a,4-Dihydroadrenochrome-3a-sulfides and Determination of Their Hemostatic Activities

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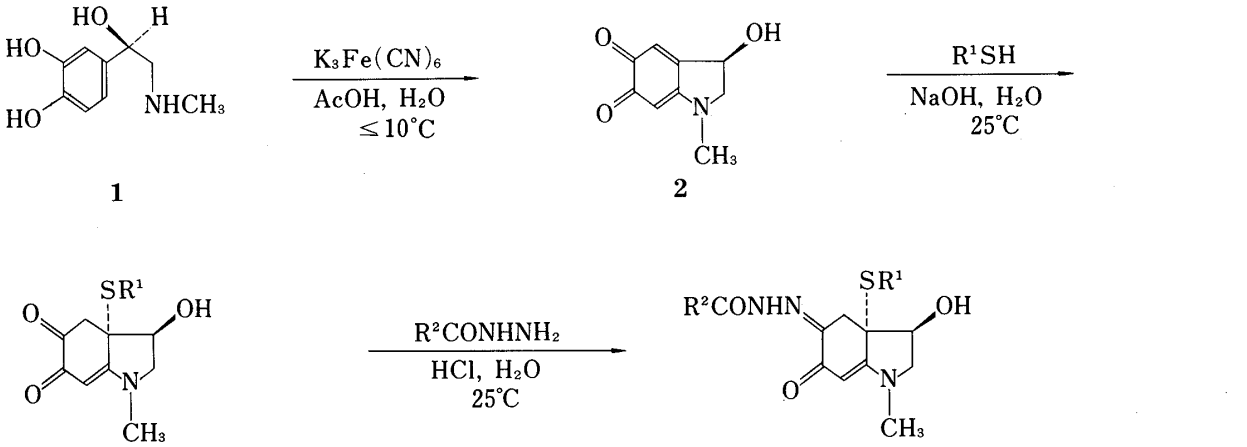
The addition reaction between thiols and adrenochrome at 25°C yielded *trans*-3a,4-dihydroadrenochrome-3a-sulfides (**3**—**5**), which were treated with hydrazides to give hydrazones (**6**—**10**). On the other hand, the reaction of adrenochrome with β-mercaptopropionic acid at 5°C, followed by treatment with benzoylhydrazine, gave *cis*-3a-(β-carboxyethylthio)-3a,4-dihydroadrenochrome monobenzoylhydrazone (**11**) as the main product. These hydrazones were tested for hemostatic effect. The hydrazone with the most potent hemostatic activity was **7**.

Keywords——addition reaction; adrenochrome; thiols; β-mercaptopropionic acid; hydrazides; *trans*- and *cis*-3a,4-dihydroadrenochrome-3a-sulfides; benzoylhydrazones; hemostatic activity

The reaction of aminochromes with thiols was first described by Green and Richter[1] in 1937. Since then, the physicochemical properties of the products obtained by this reaction and the mechanism of the reaction have been investigated by several workers.[2] Heacock and co-workers[3,4] showed that aminochromes, such as adrenochrome, reacted with thiols to give, in general, three main types of products: (A) 5,6-dihydroxyindole, (B) 5,6-dihydroxyindol-4-yl sulfides, and (C) 3a,4-dihydroadrenochrome-3a-sulfides (Michael addition products). They also suggested the possibility that suitable thiols could act as "aminochrome carriers" for the highly reactive aminochromes in physiological systems.[4] According to this assumption, hydrazone derivatives of 3a,4-dihydroadrenochrome-3a-sulfides can be expected to act



1

2

3 : $R^1=CH_2CO_2H$
4 : $R^1=CH_2CH_2CO_2H$
5 : $R^1=CH(CH_3)CONHCH_2CO_2H$

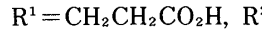
6 : $R^1=CH_2CO_2H$, $R^2=C_6H_5$
7 : $R^1=CH_2CH_2CO_2H$, $R^2=C_6H_5$
8 : $R^1=CH(CH_3)CONHCH_2CO_2H$, $R^2=C_6H_5$
9 : $R^1=CH_2CH_2CO_2H$, $R^2=NH_2$
10 : $R^1=CH_2CH_2CO_2H$, $R^2=$ 

Chart 1

TABLE I. Spectral and Analytical Data for Hydrazone Derivatives of 3a,4-Dihydroadrenochrome-3a-sulfides Obtained at a Reaction Temperature of 25 °C

Compd.	Yield (%)	mp (dec.) (°C) (solv.)	Formula	Analysis (%) Calcd (Found) C	H	N	UV $\lambda_{max}^{0.1M\ NaHCO_3}$ nm (log ε)	IR ν_{max}^{Nujol} cm^{-1}	NMR[a] δ ppm (C_5D_5N) J=Hz 3-H	Others
(±)-**6**	32	126—126.5 (MeOH)	$C_{18}H_{19}N_3O_5S\cdot CH_3OH$	54.15 (54.04	5.50 5.66	9.97 9.92)	230(4.16), 283(4.00), 369(4.41)	1715 (CO_2H), 1653	4.98[b] (d, J=3.5)	2.85 (3H, s, CH_3), 3.59 and 4.15 (2H, AB-q, J=15, 4-H_A and H_B), 3.73 (1H, d, J=12.5, 2-H_B), 3.94 (2H, s, CH_2), 4.47 (1H, dd, J=3.5, 12.5, 2-H_A), 5.64 (1H, s, 7-H), 6.64 (2H, s, OH and NH), 7.34—8.32 (5H, m, C_6H_5), 15.95 (1H, br s, CO_2H)
(±)-**7**	52	194—196 (MeOH)	$C_{19}H_{21}N_3O_5S$[c]	56.56 (56.32	5.25 5.13	10.42 10.38)	229(4.11), 284(3.97), 369(4.41)	1705 (CO_2H), 1663	4.69 (d, J=3.5)	2.76 (2H, t, J=7, CH_2), 2.77 (3H, s, CH_3), 3.16 (2H, t, J=7, CH_2), 3.31 and 4.00 (2H, AB-q, J=15, 4-H_A and H_B), 3.61 (1H, d, J=12, 2-H_B), 4.26 (1H, dd, J=3.5, 12, 2-H_A). 5.51 (1H, s, 7-H, 7.20—8.12 (5H, m, C_6H_5), 10.77 (2H, br s, OH and NH), 15.80 (1H, br s, CO_2H)
(−)-**7**	36[d]	157—159 (MeOH)	$C_{19}H_{21}N_3O_5S\cdot 3/2\ H_2O$	53.01 (53.28	5.62 5.41	9.76 9.54)	229(4.13), 283(4.01), 369(4.39)	1728 (CO_2H), 1679	4.81 (d, J=3.5)	2.76 (2H, t, J=7, CH_2), 2.77 (3H, s, CH_3), 3.16 (2H, t, J=7, CH_2), 3.31 and 4.00 (2H, AB-q, J=15, 4-H_A and H_B), 3.56 (3H, s, 3/2H_2O), 3.61 (1H, d, J=12, 2-H_B), 4.26 (1H, dd, J=3.5, 12, 2-H_A), 5.51 (1H, s, 7-H), 7.20—8.12 (5H, m, C_6H_5), 10.77 (2H, br s, OH and NH), 15.80 (1H, br s, CO_2H)
(±)-**8**	23	206—209 (DMF–H_2O)	$C_{21}H_{24}N_4O_6S$	54.77 (54.54	5.25 5.42	12.17 11.97)	230(4.14), 283(3.97), 369(4.38)	1739 (CO_2H), 1660	4.86 (d, J=3)	1.67 (3H, d, J=7, CH_3), 2.83 (3H, s, CH_3), 3.54 and 4.08 (2H, AB-q, J=16, 4-H_A and H_B), 3.70 (1H, d, J=12, 2-H_B), 4.15 (1H, q, J=7, CH), 4.45 (1H, dd, J=3, 12, 2-H_A), 4.78 (2H, d, J=6, CH_2), 5.62 (1H, s, 7-H), 5.86 (2H, br s, OH and NH), 7.28—8.20 (5H, m, C_6H_5), 9.04 (1H, t, J=6, NH), 15.95 (1H, br s, CO_2H)
(±-**9**)	30	192—194 (DMF–H_2O)	$C_{13}H_{18}N_4O_5S$	45.61 (45.60	5.30 5.38	16.36 15.96)	239(3.92), 282(3.90), 350(4.34)	1719 (CO_2H), 1700	4.48[e] (d, J=4)	2.41 (2H, t, J=6.5, CH_2), 2.86 (2H, t, J=6.5, CH_2), 3.12 (3H, s, CH_3), 3.13 and 3.35 (2H, AB-q, J=17, 4-H_A and H_B), 3.66 (1H, d, J=13, 2-H_B), 4.35 (1H, dd, J=4, 13, 2-H_A), 5.46 (1H, s, 7-H)
(±)-**10**	21	170—172.5 (EtOH)	$C_{22}H_{32}N_4O_6S$	54.98 (54.63	6.71 7.04	11.66 11.19)	253(3.94), 281(3.93), 362(4.33)	1725 (CO_2H), 1661	4.73 (d, J=3)	0.74—2.37 (10H, m, C_6H_{10}), 2.10 (3H, s, CH_3), 2.73 (3H, s, CH_3), 2.91 (2H, t, J=6.5, CH_2), 3.03—3.46 (5H, m, 2× CH_2 and 4-H_B), 3.64 (1H, d, J=12, 2-H_B), 3.94 (1H, d, J=15.5, 4-H_A), 4.33 (1H, dd, J=3, 12, 2-H_A), 5.46 (1H, s, 7-H), 8.29 (1H, t, J=6, NH), 8.79 (2H, br s, OH and NH), 14.43 (1H, br s, CO_2H)
(±)-**11**	35[f]	146—147 (MeOH)	$C_{19}H_{21}N_3O_5S\cdot CH_3OH$	55.16 (55.16	5.79 5.76	9.65 9.59)	232(4.15), 284(3.93), 371(4.39)	1700 (CO_2H), 1646	4.59[b] (t, J=8)	2.75 (3H, s, CH_3), 2.87 (2H t, J=6.5, CH_2), 3.15 and 3.67 (2H, AB-q, J=16, 4-H_A and H_B), 3.40 (2H, t, J=6.5, CH_2), 3.54 (1H, dd, J=8, 11, 2-H_B), 3.87 (1H, dd, J=8, 11, 2-H_A), 5.47 (1H, s, 7-H), 7.17—8.14 (5H, m, C_6H_5), 10.00 (2H, br s, OH and NH), 15.70 (1H, br s, CO_2H)

a) Abbreviations: s=singlet, d=doublet, dd=doublet of doublets, t=triplet, m=multiplet, br=broad. *b*) Precipitate obtained by acidification of a solution of the crystals in 2% $NaHCO_3$ with 1 N HCl was used in order to prevent overlapping of signals attributable to MeOH. *c*) S: Calcd, 7.95%, Found, 7.93%. *d*) Optical rotation of the product obtained from (*R*)-epinephrine: $[\alpha]_D^{21}$ −19° (*c*=0.22, MeOH). *e*) Measured in Na_2CO_3–D_2O. *f*) Obtained at a reaction temperature of 5°C.

as prodrugs of adrenochrome. We therefore synthesized these hydrazone derivatives and examined their biological properties. Furthermore, we found that this Michael addition reaction gave different products at different reaction temperatures, and we showed that these products have different configurations at position 3a of the adrenochrome skeleton.

Adrenochrome (**2**) was prepared by oxidation of epinephrine (**1**) with potassium ferricyanide in aqueous solution. Treatment of this adrenochrome solution with an excess of thiols such as β-mercaptopropionic acid ($R^1=CH_2CH_2CO_2H$), thioglycolic acid ($R^1=CH_2CO_2H$) or tiopronin ($R^1=CH(CH_3)CONHCH_2CO_2H$) at 25 °C in the range of pH 5.5—6.0 gave adrenochrome-thiol addition products, 3a,4-dihydroadrenochrome-3a-sulfides (**3**—**5**). The addition product (**4**) with β-mercaptopropionic acid exhibited an absorption maximum at 354 nm in the reaction solution (*e.g.*, pH 5.7), while that of adrenochrome at 488 nm disappeared after about 30 minutes. These results are in agreement with those of Powell and Heacock[3h] that the absorbance at 357 nm was highest at pH 5.9. The addition products (**3**—**5**) were treated successively with benzoylhydrazine, semicarbazide and *trans*-4-(acetamidomethyl)-cyclohexylcarbonylhydrazine in the range of pH 3.5—4.0 to give hydrazones (**6**—**10**). Intermediates were not isolated because they decomposed in the course of isolation. When the reaction with these hydrazides was carried out in the range of pH 4.2—6.0, the yields of the hydrazones (**6**—**10**) decreased with increasing production of adrenochrome monohydrazones.[5] This phenomenon can be accounted for by the reaction of hydrazides with adrenochrome which was regenerated by the undesired elimination of thiols from the addition products (**3**—**5**). The yields, spectral and analytical data for the hydrazones (**6**—**10**) are shown in Table I.

Although addition reaction with β-mercaptopropionic acid at above 20 °C, as described previously, gave only **7**, the reaction below 10 °C gave **11** as the main product; this is a diastereoisomer of **7**. At 10—20 °C, this reaction gave a mixture of **7** and **11**. The yield of **7** was lower at above 30 °C than at 20—30 °C owing to the formation of dark brown substances. The yields of **7** and **11** at these temperatures are shown in Table II. Compounds **7** and **11** were separated by treatment with boiling methanol, and relative configurations of the 3 and 3a carbons of **7** and **11** were assigned on the basis of their nuclear magnetic resonance (NMR) spectra. The NMR spectrum of **7** exhibited a doublet ($J=3.5$ Hz) at 4.69 ppm attributable to H_3 on C_3, while this proton of **11** gave a triplet signal ($J=8$ Hz) at 4.59 ppm. In spite of

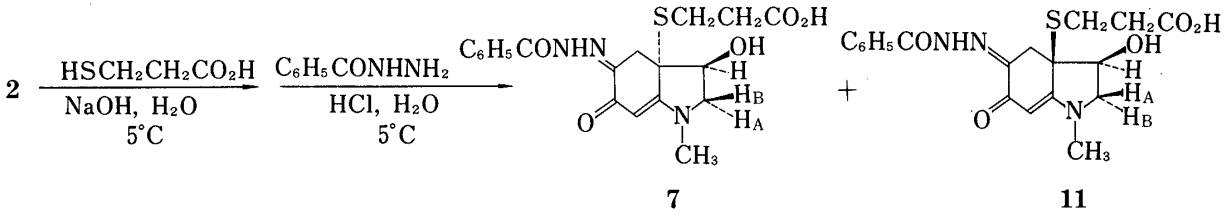


Chart 2

Table II. Benzoylhydrazones of Reaction Products of (±)-Adrenochrome with Thiols (R^1SH) at Various Temperatures

R_1	Reaction temp. (°C)	Reaction time (min)	Products (yield, %)	
CH_2CO_2H	5	30	**6**(9),	**14**(42)
CH_2CO_2H	25	30	**6**(32),	**14**(7)
$CH_2CH_2CO_2H$	5	30	**7**(19),	**11**(35)
$CH_2CH_2CO_2H$	10—15	30	**7**(25),	**11**(25)
$CH_2CH_2CO_2H$	15—20	30	**7**(34),	**11**(5)
$CH_2CH_2CO_2H$	20—25	30	**7**(48)	
$CH_2CH_2CO_2H$	30—35	15	**7**(45)	
$CH_2CH_2CO_2H$	40—45	15	**7**(18)	

the presence of an ABX system consisting of H_{2A}, H_{2B} and H_3, coupling between H_{2B} and H_3 was not observed for **7**, because the corresponding dihedral angle was about 90 ° on a molecular model. Therefore, the results indicate that the hydroxyl group on C_3 and the carboxyalkylthio moiety on C_{3a} are *trans* to one another in **7** and *cis* in **11**. It can be concluded that **6**, **8**, **9** and **10** are all *trans* isomers, since their NMR spectra showed the same pattern as that of **7** (Table I). In order to obtain optically active **7** and **11**, the reaction of β-mercaptopropionic acid with (*R*)-adrenochrome derived from (*R*)-epinephrine was attempted at temperatures of 5 °C and 25 °C. In the reaction at 25 °C (—)-(3*S*, 3a*S*)-3a-(β-carboxyethylthio)-3a,4-dihydroadrenochrome monobenzoylhydrazone (**7**) was obtained (Table I), while the reaction at 5 °C gave (—)-**7** and (+)-(*R*)-adrenochrome monobenzoylhydrazone (**14**)[6] (yields of 15% and 11%, respectively). The reason why optically active **11** could not be isolated is unclear.

The addition reaction of adrenochrome with thioglycolic acid at 5 °C, followed by reaction with benzoylhydrazine, gave **6** in low yield (Table II); the *cis* isomer corresponding to **11** was so unstable that it decomposed to adrenochrome monobenzoylhydrazone (**14**) in the course of isolation. The compound **11** showed rather poor thermostability: heating of the sodium salt of **7** in aqueous solution at 60 °C for 24 h resulted in scarcely any elimination of β-mercaptopropionic acid, while the sodium salt of **11** decomposed on heating under comparable conditions to give **14** in 10% yield. These results and the foregoing relationship between pH and yields of the hydrazones can be interpreted by the following mechanism: the less stable addition product (**13**) is formed under kinetic control at lower temperature and **13** is converted *via* adrenochrome (**2**) to the more stable addition product (**12**) under thermodynamic control at higher temperature (Chart 3). The compound **6** or **8** was less stable than **7** and gradually decomposed in aqueous sodium bicarbonate solution at room temperature to give crystals of **14** after a few days.

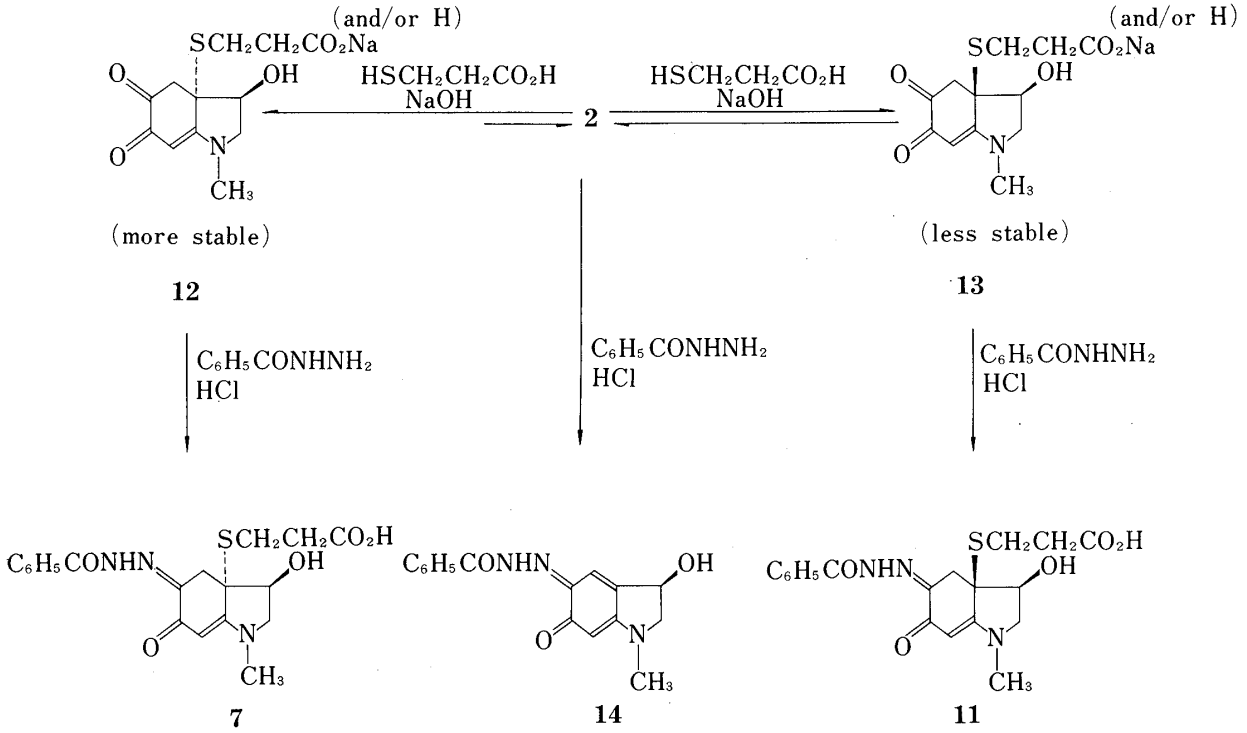


Chart 3

The benzoyl hydrazones (**6**, **7**, **8** and **11**) were tested for activity to shorten the bleeding time upon cutting off a mouse tail, for depressive activity on pulmonary hemorrhage in mice under reduced pressure,[7] and for inhibitory effect against accelerated permeability induced

Table III. Hemostatic Activities of Benzoyl Hydrazone Derivatives of 3a,4-Dihydroadrenochrome-3a-sulfides

Compd.	Bleeding time	Capillary resistance	Capillary permeability
15	1.0[a]	1.0[a]	1.0[a]
(±)- **6**	0.9	—	—
(±)- **7**	1.0	2.5	1.0
(±)- **8**	0.7	2.0	0.6
(±)-**11**	0	—	0.6

a) The hemostatic activties of carbazochrome sodium sulfonate (**15**) (5 mg/kg, *s.c.*) were taken as 1.0.

by histamine in rat skin.[8] The results are shown in Table III, along with comparative data for carbazochrome sodium sulfonate (**15**).[9]

The most potent benzoylhydrazone was **7** which was 2.5 times as active as **15** in the capillary resistance test. Compounds **6** and **8** showed hemostatic activity comparable with that of **15** in these tests, while the *cis* isomer (**11**) of **7** showed lower activity. It is therefore assumed that adrenochrome-thiol addition products *in vivo* are also in the stable *trans* form.

Experimental

Melting points were determined on a Yamato MP-21 melting point apparatus and are uncorrected. Measurements of pH were made with a Hitachi-Horiba H-5 pH meter. Specific rotations were measured with a JASCO DIP-4 polarimeter. Infrared (IR) spectra were recorded on a JASCO IR-E spectrophotometer. Ultraviolet (UV) spectra were obtained with a Hitachi 239 spectrophotometer. NMR spectra were measured on a JEOL PS-100 spectrometer using tetramethylsilane or 3-(trimethylsilyl)propanesulfonic acid sodium salt as an internal standard. All chemicals were reagent grade commercial materials and were used without further purification. ADONA® (AC-17) injection (Tanabe) was used for biological tests as carbazochrome sodium sulfonate (**15**).

(±)-***trans*-3a-(β-Carboxyethylthio)-3a,4-dihydroadrenochrome Monobenzoylhydrazone** (**7**)——A solution of potassium ferricyanide (39.5 g, 0.12 mol) and sodium bicarbonate (10 g, 0.119 mol) in 180 ml of water was slowly added to a solution of (±)-epinephrine (5.5 g, 0.03 mol) and glacial acetic acid (2.4 ml, 0.042 mol) in 30 ml of water at a temperature below 10°C with stirring. After 5 min of stirring at 5°C, a solution of β-mercaptopropionic acid (6.3 g, 0.059 mol) in 27 ml of 2 N NaOH was added to the mixture all at once. After the addition, stirring was continued for 30 min at 25°C and then a solution of benzoylhydrazine (4.1 g, 0.03 mol) in 58 ml of 2 N HCl was added to the reaction mixture. The whole was allowed to stand overnight in a refrigerator and decanted to remove the aqueous solution. Methanol (30 ml) was added to the residual gummy material, and stirring was continued until the gum crystallized. The resulting orange crystals were collected and dissolved in 80 ml of 2.5% $NaHCO_3$. After filtration, the filtrate was slowly acidified with 1 N HCl. The precipitate was filtered off, dried, and recrystallized from methanol to afford **7** (5.8 g, 52%) as yellowish-orange prisms.

The other benzoylhydrazones (**6**, **8**) and (−)-**7** was obtained in a similar fashion.

(±)-***cis*-3a-(β-Carboxyethylthio)-3a,4-dihydroadrenochrome Monobenzoylhydrazone** (**11**)——An ice-cooled solution of β-mercaptopropionic acid (6.3 g, 0.059 mol) in 27 ml of 2 N NaOH was added to an adrenochrome solution prepared from (±)-epinephrine (5.5 g, 0.03 mol) by the above procedure with stirring. The reaction mixture was stirred for 30 min at 5°C, then a solution of benzoylhydrazine (4.1 g, 0.03 mol) in 58 ml of 2 N HCl was added and the whole was allowed to stand overnight in a refrigerator and decanted to obtain the gummy material. The residual gum was crystallized by stirring in 30 ml of methanol. The resulting orange crystals were collected, and dissolved in 85 ml of 2.5% $NaHCO_3$. After filtration, the filtrate was very slowly acidified with 0.5 N HCl. The precipitate was filtered off, dried, and added to 150 ml of boiling methanol. The suspension was refluxed for 5 min, then filtered, and the remaining solid was recrystallized from methanol to give **11** (4.6 g, 35%) as orange needles.

The filtrate was cooled to give **7** (2.3 g, 19%) as yellowish-orange prisms.

(±)-***trans*-3a-(β-Carboxyethylthio)-3a,4-dihydroadrenochrome Monosemicarbazone** (**9**)——A solution of semicarbazide hydrochloride (3.3 g, 0.03 mol) in 33 ml of 8% formic acid was added to an addition product solution (produced by the same procedure as in the case of **7**) and the reaction vessel was rubbed with a glass rod and allowed to stand overnight. The resulting precipitate was filtered off, washed several times with 100 ml each of hot THF, and dissolved in 80 ml of 2.5% $NaHCO_3$. The solution was filtered and the filtrate

was acidified with 1 N HCl. The resulting yellowish crystals were collected, and recrystallized from DMF-water to give **9** (3.2 g, 30%) as yellowish fine crystals.

***trans*-4-(Acetamidomethyl)cyclohexylcarbonylhydrazine**——A stream of hydrogen chloride was passed through a suspension of tranexamic acid (62.9 g, 0.4 mol) in 150 ml of dry methanol under reflux for 5 h. After concentration of the resulting mixture, 300 ml of dry ether was added to the residue and the separated colorless prisms were filtered off to give methyl tranexamate hydrochloride (79.4 g, 96%), mp 204—211°C (lit.[10] mp 168—170°C).

Acetic anhydride (51 ml, 0.54 mol) was added dropwise to a solution of this compound (62.3 g, 0.3 mol) and $NaHCO_3$ (84 g, 1.0 mol) in 400 ml of water with stirring. The resulting mixture was allowed to stand overnight in a refrigerator, and the separated colorless needles were filtered off to give methyl N-acetyltranexamate (50.9 g, 80%), mp 81—84°C.

A solution of this product (48.9 g, 0.23 mol) and hydrazine hydrate (24 ml, 0.49 mol) in 100 ml of dry ethanol was refluxed for 4 h, then concentrated. The resulting crystals were collected and recrystallized from 90% ethanol to give *trans*-4-(acetamidomethyl)cyclohexylcarbonylhydrazine (33.8 g, 69%) as a white powder, mp 214—215°C, IR ν_{max}^{Nujol} cm^{-1}: 3370 (NH), 1630 (CONH).

(±)-*trans*-3a-(β-Carboxyethylthio)-3a,4-dihydroadrenochrome Mono[*trans*-4-(acetamidomethyl)cyclohexylcarbonyl]hydrazone (10)——A solution of *trans*-4-(acetamidomethyl)cyclohexylcarbonylhydrazine (6.4 g, 0.03 mol) in 60 ml of 2 N HCl was added to an addition product solution (produced by the same procedure as in the case of **7**). After standing overnight in a refrigerator, the resulting gum was separated from the reaction solution by decantation and crystallized from 10 ml of ethanol containing a small amount of methanol. The orangeish crystals were filtered off and recrystallized from ethanol to give **10** (3.0 g, 21%) as orangeish fine crystals.

Determination of Bleeding Time——Male ddy mice weighing 25 to 30 g were used. The animals were placed in a plastic holder with holes for ventilation. The tail was cut off 1.5 cm from the tip with a razor, and was allowed to bleed into a beaker containing 100 ml of deionized water at 25°C. The tail was observed until bleeding stopped. Test drugs were administered subcutaneously 30 min before cutting. The shortening effects of the drugs on the bleeding time were expressed as the relative activity with respect to **15** (5 mg/kg, *s.c.*).

Determination of Capillary Resistance——Male ddy mice weighing 14 to 19 g were used. Pulmonary hemorrhage in these mice was estimated as a parameter of capillary resistance. The animals were placed in a reduced pressure (-43 ± 1 mmHg) box for 30 s, and the intensity of pulmonary hemorrhage was determined by measuring the specific gravity of the lung. Test drugs were administered subcutaneously 15 min before the experiment, and the inhibitory effects were expressed as the relative activity with respect to **15** (5 mg/kg, *s.c.*).

Determination of Capillary Permeability——Male Wistar rats weighing 170 to 200 g were used. An increase of capillary permeability in the rat skin was caused by intradermal injection of histamine (10μg). At 10 min later the extent of increased capillary permeability was estimated by measuring the amount of dye that had leaked into the skin; the dye was injected intravenously just prior to the administration of histamine. Test drugs were administered subcutaneously 20 min before the histamine injection. The inhibitory effects of the drugs on the capillary permeability were expressed as the relative activity with respect to **15** (5 mg/kg, *s.c.*).

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