RADIOLYSIS AND SYNTHESIS OF [N-METHYLENE-^{3}H]THIAMINE 1-ADAMANTYL TRISULFIDE HYDROCHLORIDE

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SUMMARY

[N-methylene-^{3}H]Thiamine 1-adamantyl trisulfide hydrochloride (VII) was synthesized. Both the γ-radiolysis of thiamine 1-adamantyl trisulfide hydrochloride (VIII) in ethanol and the self-radiolysis of VII in the crystalline state containing 17 mole-percent of ethanol have been studied. G(-VII) in the crystalline state by the gross effects of self-irradiation was 3.4, and the four decomposition products were found. The self-radiolysis products yielded from VII in the crystalline state were the same as those products isolated from the γ-radiolysis of VIII in ethanolic solution and determined the structures as di-(1-adamantyl)tetrasulfide, 2-dihydro-3-(4-amino-2-methyl-5-pyrimidinylmethyl)-5-(1-adamantyl dithio)-3a-methylperhydrofuro[2,3-d]thiazole, 2-(1-hydroxyethyl)-3-(4-amino-2-methyl-5-pyrimidinylmethyl)-5-(1-adamantyl dithio)-3a-methylperhydrofuro-[2,3-d]thiazole and thiamine chloride hydrochloride respectively.

Key Words: Synthesis, [N-methylene-^{3}H]Thiamine 1-adamantyl trisulfide hydrochloride, Radiolysis products

INTRODUCTION

Since allithiamine was found in 1952[1], many derivatives of thiamine thiol-form have been synthesized for the investigation of their biological activity[2,3]. The tritium labelled compound of thiamine 1-adamantyl trisulfide hydrochloride (VIII)[4] with high specific activity was desired for the study of metabolic fate in animals. It has been recognised that many compounds labelled with

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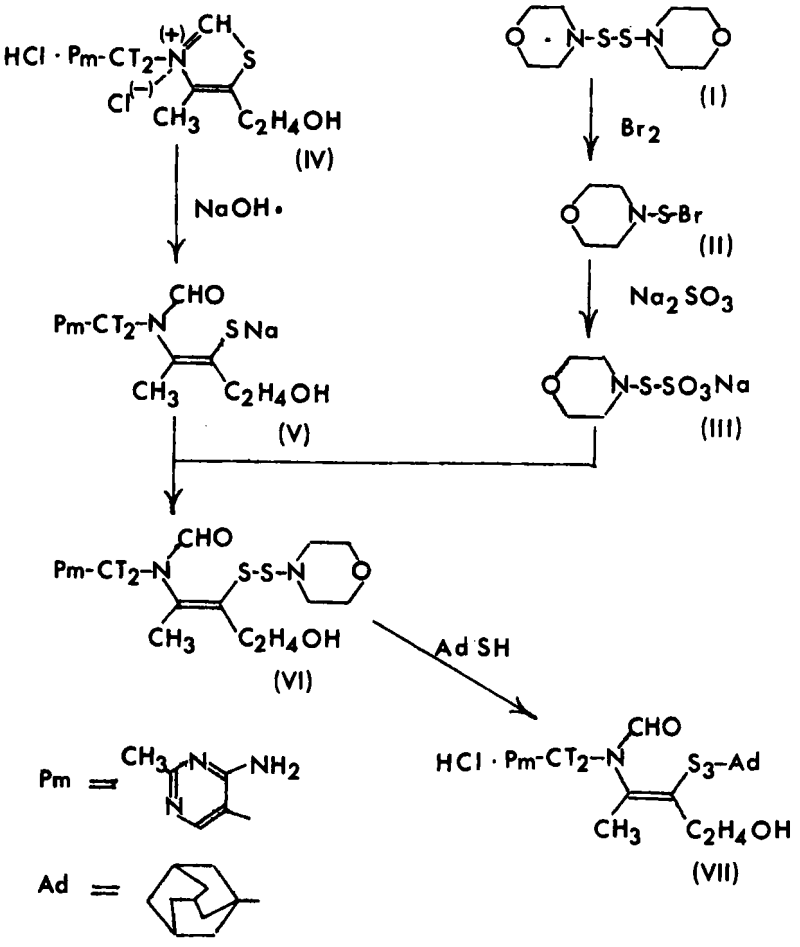
radioisotopes, especially tritium labelled compounds with high specific activity, decompose on storage by the almost complete absorption of the beta-radiation energy and that the decomposition is accelerated by self-irradiation[5]. In view of the considerable interest in the radiolysis of VIII this paper deals with the synthesis of [N-methylene-^{3}H]thiamine 1-adamantyl trisulfide hydrochloride (VII), the extent of radiation decomposition and the determination of product structure in order to provide data which will assist in a more scientific evaluation on the radiolysis by self-irradiation.

RESULTS

Synthesis of VII

Morpholinosulfenyl bromide (II) prepared by the bromination of 4,4-dithiodimorpholine (I) was treated with sodium sulfite to give sodium morpholino thiosulfate (III). [N-methylene-^{3}H]Thiamine morpholino disulfide (VI) was prepared by the condensation of III with thiol-form of [N-methylene-^{3}H]thiamine (V). VI was reacted with adamantane-thiol to afford VII in 59% radiochemical yield based on [N-methylene-^{3}H]thiamine chloride hydrochloride (IV). The synthetic route of VII is shown in Scheme 1.

Scheme 1


HCl · Pm-CT2-N
CH
S
Cl(−)
CH3
C2H4OH
(IV)
NaOH
(I)
Br2
N-S-Br
(II)
Na2SO3
N-S-SO3Na
(III)
CHO
SNa
(V)
S-S-N
(VI)
Ad SH
Pm =
NH2
Ad =
S3-Ad
(VII)

Gamma-radiolysis of VIII in ethanol

The thin-layer chromatographic examination of the irradiated ethanolic solution of VIII revealed four decomposition products at Rf. 0.96 (IX), 0.82 (X), 0.65 (XI) and 0.0 (XII) in addition to the unchanged free base of VIII respectively. The four products were separated over a column chromatogram and the mole percent yield of these products were summarised in Table 1.

Table 1. The radiolysis products of VIII in ethanol

Fraction	Yield(*) found in mg	Mole percent yield %
Unchanged free base of VIII	1620	27.0
IX	936	40.5
X	150	2.8
XI	1490	25.2
XII	1562	39.9

(*) Dose, 4.74×10^{22} eV/l

The total recovery of the products and the unchanged free base of VIII was 95%. Each structure of products was determined as follows:

Di-(1-adamantyl)tetrasulfide (IX).

The nuclear magnetic resonance (NMR) spectrum showed the characteristic absorption of the adamantane-ring protons at δ=1.65, 1.95 and 2.05 which contained 6, 6 and 3 protons respectively. No other signals were found in this spectrum. The identity of the compound was further confirmed by infra-red (IR), mass spectrum (MS, m/e= 398, molecular ion) and elemental analysis ($C_{20}H_{30}S_4$).

2-Dihydro-3-(4-amino-2-methyl-5-pyrimidinylmethyl)-5-(1-adamantyl dithio)-3a-methylperhydrofuro[2,3-d]thiazole (X).

The ultra-violet (UV) spectrum [λ_{max}^{EtOH} nm (ε): 234 (1.06×10^4) and 275 (0.63×10^4)] resembled to that of

dihydrothiamine[6] and suggested that the pyrimidine nucleous might be a mono-cyclic system. The NMR spectrum in $CDCl_3$ showed the characteristic absorption of the adamantane-ring protons, and the absorptions at $\delta = 2.48$ (s, 3H), 7.91 (s, 1H), 3.69 (s, 2H), 1.54 (s, 3H) and 5.94 (b, 2H; disappeared in D_2O) indicated the presence of the pyrimidine methyl, the proton at 6 in the pyrimidine, 5-pyrimidinylmethyl, 3a-methyl of thiazole and a primary amino group respectively. The signals in the region from $\delta = 2.16$ to 4.15 were checked by the spin-decoupling method and attributed to the β-protons [2.16 (m, 1H) and 2.90 (m, 1H)], the α-protons in the perhydrofuro ring [4.47 (m, 2H)], and the protons at 2 in the thiazole [3.51 (d, 1H; J=14 Hz) and 4.08 (d, 1H; J=14 Hz)] respectively. No signal of N-formyl proton at $\delta = 8.08$ was found. The MS (m/e = 464, molecular ion) and the elemental analysis were identical to that of X as $C_{22}H_{32}ON_4S_3$. Reaction of X with anhydrous MeOH-HCl provided a product identical with an authentic thiamine chloride hydrochloride.

2-(1-Hydroxyethyl)-3-(4-amino-2-methyl-5-pyrimidinylmethyl)-5-(1-adamantyl dithio)-3a-methylperhydrofuro[2,3-d]thiazole (XI).

The NMR spectrum of XI in $CDCl_3$-CD_3OD mixture (4:1, v/v) showed the presence of adamantane, and the absorptions at $\delta = 1.28$ (s, 3H), 2.45 (s, 3H), 1.19 (d, 3H) and 7.93 (s, 1H) were supported the presence of 3a-methyl of thiazole, pyrimidine methyl, the methyl of 2-(1-hydroxyethyl) and the proton at 6 in the pyrimidine respectively. The signals in the region from $\delta = 2.22$ to 4.28 were checked by the spin-decoupling method and attributed to the β-protons [2.22 (m, 1H) and 2.82 (m, 1H)] and the α-protons in perhydrofuro ring [4.02 (m, 1H) and 4.17 (m, 1H)], 5-pyrimidinylmethyl [3.90 (d, 2H)], the proton at 1 in 2-(1-hydroxyethyl) [4.10 (m, 1H)] and the proton at 2 in thiazole [4.28 (d, 1H)]. The structure of XI as $C_{23}H_{34}O_2N_4S_3$ was further confirmed on the basis

of the elemental analysis, UV, IR and MS (m/e = 508, molecular ion). Treatment of XI with anhydrous MeOH-HCl resulted in the formation of 2-(1-hydroxyethyl)thiamine chloride hydrochloride which was confirmed by the identity of its elemental analysis, IR, UV and NMR spectra with that of the literature[7].

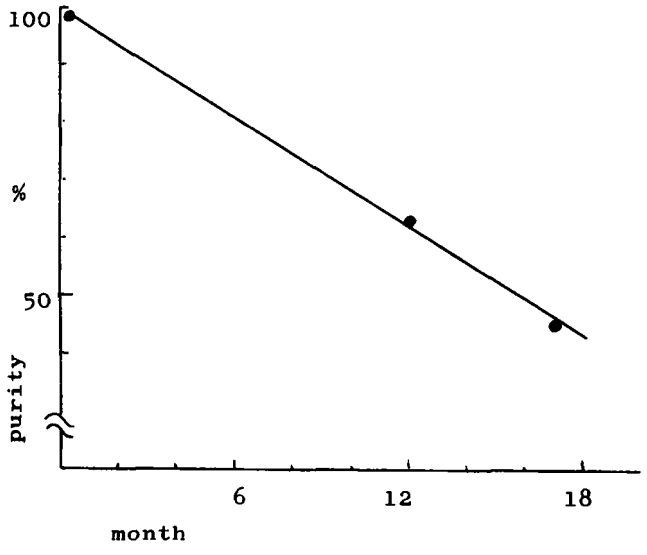
Thiamine chloride hydrochloride (XII).

The structure of XII was confirmed by the identity of its chromatographic mobility, elemental analysis, IR and NMR spectra with that of an authentic thiamine chloride hydrochloride.

Self-radiolysis of crystalline VII

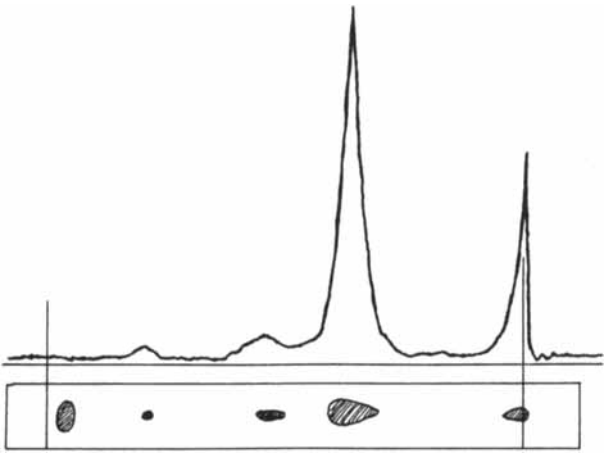
The crystals of VII with a specific activity of 1 Ci/mmole contained 17 mole-percent of ethanol which was gas-chromatographically determined and the initial radiochemical purity of VII was shown to be 98% by both the reverse isotope dilution and radiochromatographic methods. The crystals of VII were stored in a freezer at -20° in the presence of air. After 12 and 17 months' storage, the isotope dilution analysis indicated that the purity of VII at the end of those period was 63 and 45% respectively (Fig. 1).

Fig. 1. The purity percent of VII at the end of 12 and 17 months' storage.



The chromatographic examination of VII at the end of 17 months' storage using a silica gel TLC plate in a developing solvent (n-hexane, AcOEt, MeOH; 4:4:1, v/v) revealed four decomposition products at Rf. 0.96 (F-1), 0.82 (F-2), 0.65 (F-3) and 0.0 (F-4) in addition to the unchanged VII which were found radioactivity without F-1 (Fig. 2).

Fig 2. Radiochromatography of VII at the end of 17 month's storage.



Precoated TLC plate:	Silicagel-f (Tokyokasei, Ltd).
Developing solvent:	n-Hexane, AcOEt, MeOH (4:4:1, v/v).
Visualization:	Fluorescence or iodine staining.
Radiochromatogram:	Aloka TRM-1B. Full scale, 30 k cpm. Time constant, 3 sec. Slit, 1.5 mm. Scanning speed, 300 mm/h.

F-1, 2, 3 and 4 were eluted from the TLC plate. The chromatographic mobility and the retention time of the high speed liquid-chromatography of these products were identical with that of the authentic IX, X, XI and XII respectively.

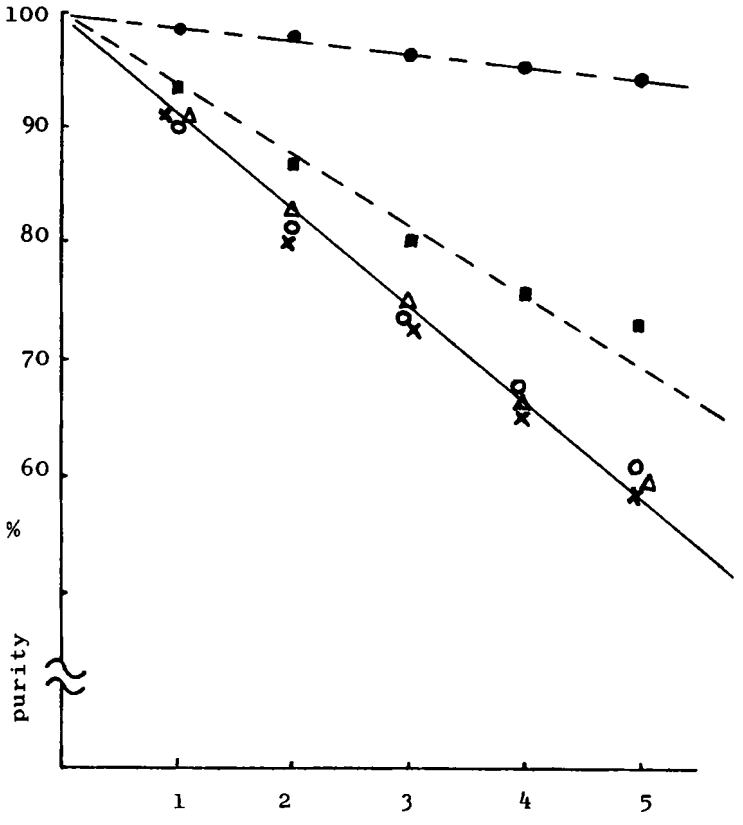
DISCUSSION

Self-radiolysis during storage can be roughly divided into two mode of decomposition which are expressed by the internal primary effect and the external radiation effect[8]. The decomposition due to the internal primary decay of tritium to helium over the period of time in this experiment was less than 0.18% per annum by the calculation. The actual decomposition products of VII in the crystalline state will therefore arise through external radiation effect. Based on the decomposition rate of VII in Fig. 1, G(-VII) by the gross effects of self-irradiation was computed to be 3.4. When dilute ethanolic solutions are irradiated, practically all the energy absorbed is deposited in solvent molecules and the observed chemical changes are brought about indirectly via the radical intermediate, i.e. solvated electrons, hydrogen atoms and α-hydroxyethane radicals as the reactive species[9]. The decomposition rate of VIII and the free base of VIII in ethanolic solution with γ-radiation in the presence and absence of scavengers were measured by means of a high speed liquid chromatography and the results are presented in Fig. 3.

G(-free base of VIII) = 3.2 in an argon saturated solution decreases with increasing dose within the range of 3.6×10^{21} to 18×10^{21} eV/l. But the difference between G-values in the argon saturated solution and in the presence of nitrous oxide as scavenger of solvated electrons is 0.8, and this value is similar to the G-yield of solvated electrons in ethanol[10]. Nitrous oxide has little or

no effect on G(-VIII) within the experimental error, and this may be the reason that HCl salt scavenges the solvated electron and the electron is converted to hydrogen atom. On the other hand, the decomposition rate of VIII is apparently decreased by the addition of oxygen as the scavenger of the solvated electrons and hydrogen atoms. The evidence indicates that the major species of ethanol radiolysis, solvated electrons and hydrogen atoms, partici-

Fig 3. Decomposition rate of VIII



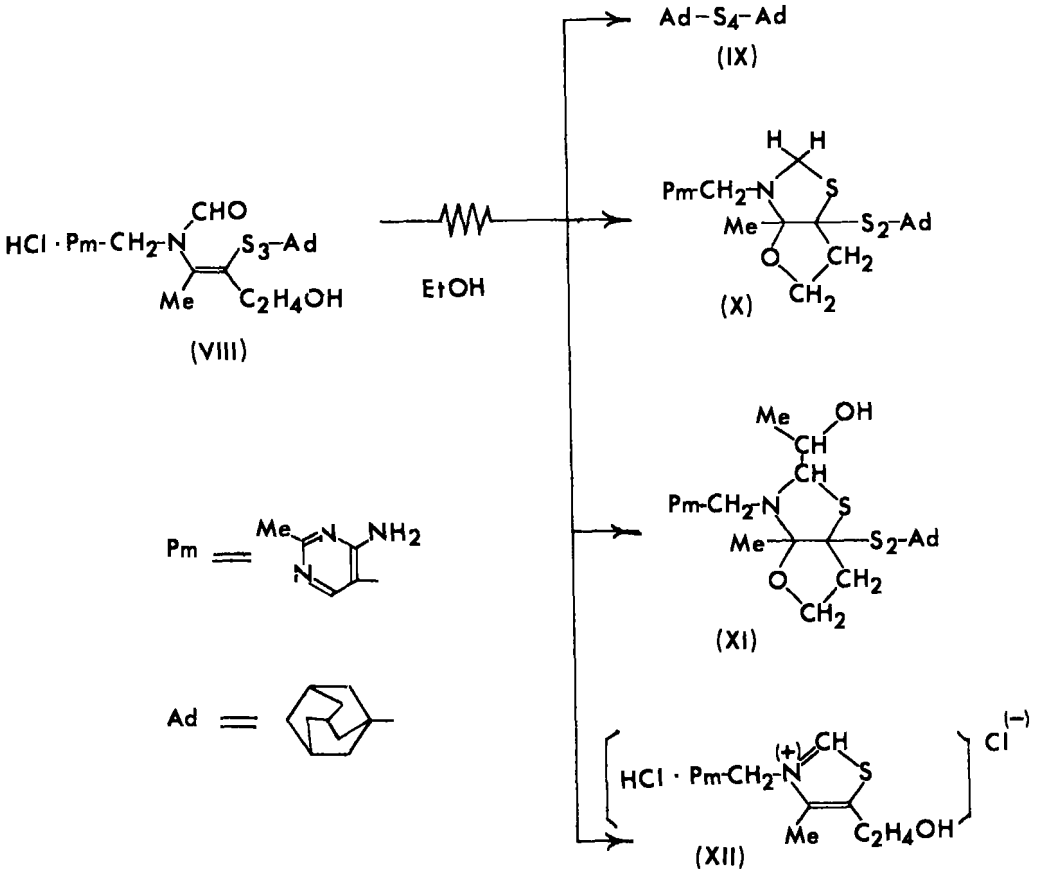
free base of VIII/Ar(—o—), VIII/Ar(—x—)

free base of VIII/N_2O(--■--), VIII/N_2O(—△—)

free base of VIII/O_2 (—·●—).

pate in the decomposition of VIII. Rupture of the disulfide linkage has been successfully correlated with the selective attack of hydrogen atoms and solvated electrons at disulfide bridges in enzymes[11] and organic disulfides[12], so that the decomposition of VIII will initially proceed through a trisulfide bond rupture and then IX, X, XI and XII are formed.

Reaction scheme 2



Direct action with ionizing radiation may be significant in solid state, but the self-decomposition products yielded from VII in the crystalline state containing 17 mole-percent of ethanol were the same as each product isolated from the γ-radiolysis of VIII in ethanolic solution. By analogy with the effect of water of

crystallization on the radiolysis of α-sulfobenzyl penicillin[13], the significant effect of solvent of crystallization is discernible from the fact that ethanol is responsible for the formation of XI on the radiolysis in the crystalline state. Both hydrogen atoms and electrons are able to diffuse through the solid when the ice is irradiated at the temperature above about -50°[14]. Therefore, the interpretation of the results is somewhat speculative, but the mechanism is probably similar to that occurring in the solution. The details mechanism will be discussed elsewhere.

EXPERIMENTAL

Material

[N-methylene-^{3}H]Thiamine chloride hydrochloride was purchased from The Radiochemical Centre, Amersham, England.

Sodium morpholino thiosulfate (III)

To a mixture of 260 mg (1.1 mmole) of 4,4'-dithiodimorpholine and 0.4 ml of CCl_4, 176 mg (1.1 mmole) of bromine in 1.1 ml of CCl_4 was added dropwise below 0° with stirring for 10 min. After being filtered, the filtrate was added to a solution of 276 mg of Na_2SO_3 in 1.1 ml of water, and the resulting solution was stirred for 30 min in an ice bath. The aqueous upper-layer was washed with a small amount of ether, and the aqueous solution of III was then obtained.

[N-methylene-^{3}H]Thiamine morpholino disulfide (VI)

Into a solution of 1.6 ml of 7.5% NaOH, 337 mg (1.1 Ci/mmole) of [N-methylene-^{3}H]thiamine chloride hydrochloride was dissolved and the solution was allowed to stand for 30 min at room temperature. To the resulting solution (V), an aqueous solution of III was added with stirring for 2 h at room temperature. The crystalline product (233 mg of VI) was filtered off and washed with a small amount of cold water, mp 175°, Rf. 0.57 on TLC (silica gel

spot film (Tokyokasei, Ltd.), developing solvent; acetone, benzene and methanol, 2:2:1, v/v).

[N-methylene-^{3}H]Thiamine 1-adamantyl trisulfide (VII)

To a solution of 232.7 mg of VI in 12 ml of ethanol, 117 mg of adamantane thiol in a mixture of 0.24 ml of 5N HCl-EtOH and 2.4 ml of ethanol was added dropwise and stirring for 1 h at room temperature. The resulting mixture was filtered and then the filtrate was evaporated to dryness under reduced pressure. To the residue, petroleum ether was added with stirring. The crystalline product was filtered off, washed with a small amount of cold ethanol then cold water and dried in vacuo. Recrystallization from ethanol gave 649 mCi of VII with a specific activity of 1056.7 mCi/mmole, mp 185-187° (dec), Rf. 0.72 on TLC (silica gel spot film (Tokyokasei, Ltd.), developing solvent; acetone, benzene and methanol, 2:2:1, v/v), identical with that of the authentic sample. The yield was 59% based on [N-methylene-^{3}H]thiamine chloride hydrochloride.

γ-Radiolysis of thiamine 1-adamantyl trisulfide hydrochloride (VIII) in ethanol

A solution of 6 g of VIII in 6 L of ethanol was swept with argon for 1 h and irradiated at room temperature to a dose of 4.74×10^{22} eV/l at a dose rate of 2.37×10^{22} eV/l.h. The irradiated solution was evaporated to 1/100 of its volume under reduced pressure and the residue was kept in a refrigerator. The crystalline product (IX) was removed by filtration and then the filtrate was concentrated to half of its volume in vacuo. The second crude product (XII) precipitated as a crystalline solid was filtered off. The final filtrate was chromatographed over a silica gel column (400 g, Ø:·0.063-0.2 mm, Merck) and eluted with a mixture of n-hexane, AcOEt and MeOH (2:1:1, v/v) to afford four fractions, which were concentrated and dried in vacuo to give IX, X, XI and the unchanged

free base of VIII respectively. The final fraction from the column chromatogram was eluted with a mixture of n-hexane, AcOEt and MeOH (1:2:3, v/v), and concentrated to give XII. The TLC plates (Tokyo-kasei, Ltd.) used were 20 x 20 cm spot-film precoated with silica gel, developed in n-hexane, AcOEt, MeOH (3:3:1, v/v) and visualized with UV illumination and iodine staining reaction.

Di-(1-adamantyl)tetrasulfide (IX).

Recrystallization of crude IX (936 mg) from a mixture of MeOH and $CHCl_3$ gave colourless plate, mp 134-5°. IR ν_{max}^{KBr} cm^{-1}: 2900, 2850, 1450, 1340, 1300 and 1040 (adamantane ring, CH stretching and deformation). Anal. Calcd. for $C_{20}H_{30}S_4$: C, 60.25; H, 7.85; S, 32.17. Found: C, 59.90; H, 7.21; S, 31.80. MS (m/e): 398 (M^+), 366 (M^+-S), 334 (M^+-S_2), 136 (AdH^+) and 135 (Ad^+).

2-Dihydro-3-(4-amino-2-methyl-5-pyrimidinylmethyl)-5-(1-adamantyl dithio)-3a-methylperhydrofuro[2,3-d]thiazole (X).

Recrystallization of crude X (150 mg) from methanol gave colourless needles, mp 198°. IR ν_{max}^{KBr} cm^{-1}: 3360, 3150 (NH stretching), 2900, 2850 (CH stretching in adamantane ring), 1640, 1570 (C=C in pyrimidine), 1590 (NH deformation), 1450 (CH bending) and 1020 (pyrimidine-NH_2 bending). MS (m/e): 464 (M^+), 297 (M^+-AdS), 265 (M^+-AdS_2), 136 (AdH^+) and 135 (Ad^+). Anal. Calcd. for $C_{22}H_{32}ON_4S_3$: C, 56.86; H, 6.94; N, 12.06; S, 20.70. Found: C, 56.49; H, 6.93; N, 11.93; S, 20.64.

2-(1-Hydroxyethyl)-3-(4-amino-2-methyl-5-pyrimidinylmethyl)-5-(1-adamantyl dithio)-3a-methylperhydrofuro[2,3-d]thiazole (XI).

Recrystallization of crude product XI (1490 mg) from a mixture of MeOH and $CHCl_3$ gave colourless needles, mp 205°. UV λ_{max}^{EtOH} nm (ε): 236 (1.14 x 10^4) and 278 (0.58 x 10^4). IR ν_{max}^{KBr} cm^{-1}: 3440, 3320, 3210 (NH and OH stretching), 2900, 2850 (CH stretching in adamantane ring), 1630 (C=C in pyrimidine), 1590 (NH deformation)

and 1470 (CH bending). MS (m/e): 508 (M^+), 476 (M^+-S), 463 (M^+-CH_3CHOH), 309 (M^+-AdS_2), 295 (309-CH_2), 126 (AdH^+) and 135 (Ad^+). Anal. Calcd. for $C_{24}H_{36}O_2N_4S_3$: C, 56.66; H, 7.13; N, 11.01; S, 18.91. Found: C, 56.30; H, 7.05; N, 11.00; S, 18.58.

Reaction of X with MeOH-HCl

In a mixture of 5 ml of MeOH and 3 ml of anhydrous MeOH saturated with HCl, 300 mg of X was dissolved. The solution was allowed to stand over-night at room temperature and the crystalline product was removed by the filtration. Evaporation of the filtrate left a residue which was extracted with a mixture of $CHCl_3$ and water with stirring. The crystalline product and the $CHCl_3$ layer were combined, then the solution was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was recrystallized from a mixture of $CHCl_3$ and MeOH to give 110 mg of colourless plates, mp or mixed with IX, 134-5°, which was identical in every respects with that of the authentic IX. The aqueous layer was washed with $CHCl_3$ and freeze-dried in vacuo. Recrystallization from a mixture of water, EtOH and acetone gave 80 mg of colourless needles, the identity of which was confirmed by its mp, NMR and elemental analysis with that of an authentic thiamine chloride hydrochloride.

Reaction of XI with MeOH-HCl

In a mixture of 10 ml of MeOH and 5 ml of anhydrous MeOH saturated with HCl, 670 mg of XI was dissolved. The solution was allowed to stand over-night at room temperature and the isolation procedure of products was similar to that of the reaction of X with MeOH-HCl. From the $CHCl_3$ layer, 180 mg of IX was obtained. The aqueous layer was washed with $CHCl_3$ and freeze-dried in vacuo. Recrystallization from ethanol gave 130 mg of colourless crystals, mp 226-8° (dec). IR ν_{max}^{KBr} cm^{-1}: 3450, 3250, 3050, 2800, 1660, 1620, 1110. NMR (in D_2O, σ ppm): 1.75 (d, 3H), 2.45 (s, 3H), 2.65 (s, 3H), 3.25 (t, 2H), 3.95 (t, 2H), 5.48 (m, 1H), 5.65 (s, 2H) and

7.45 (s, 1H). Anal. Calcd. for $C_{14}H_{22}O_2N_4SCl_2$: C, 44.10; H, 5.82; N, 14.69; S, 8.41; Cl, 18.59. Found: C, 43.67; H, 5.84; N, 14.80; S, 8.91; Cl, 18.49.

Gas-chromatography

In 1 ml of pyridine was dissolved 270 mg of VII, and an aliquot (2 μl) of the solution was injected into the gas-chromatogram (Model G-80, Yanako). The amount of ethanol in VII was measured and calibrated with the standard curve. The column: 1 m of chromosorb-105 (80-100 mesh), temperature: 120°, He: 1.1 kg/cm^2 (16 L/min), H_2: 0.3 kg/cm^2. The retention time of EtOH was found at 6 min 48 sec.

High speed liquid-chromatography

The retention times of IX, X and XI were measured with the high speed liquid-chromatogram (Model-841, Shimadzu, Ltd.) equipped with UV detector (254 nm) and found at 4 min 12 sec, 15 min 12 sec and 26 min 42 sec respectively. The column used was 2 mm diameter, 25 cm length of the micro-beads of silica gel (Ø, 5 μ) and the pressure was 70 kg/cm^2. A mixture of n-hexane, 1,2-dimethoxyethane and methanol (195:92:33, v/v) was used as an eluent.

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