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A NEW RADIATION-INDUCED REACTION OF THIAMINE WITH DI-1-ADAMANTYL TETRASULFIDE

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Radiation-induced reaction of thiamine monochloride with di-1-adamantyl tetrasulfide gave two products, 3-(4-amino-2-methyl-5-pyrimidinylmethyl)-6a-(1-adamantyldithio)-3a-methylperhydrofuro[2,3-d]thiazole and 2-(1-hydroxymethyl)-3-(4-amino-2-methyl-5-pyrimidinylmethyl)-6a-(1-adamantyldithio)-3a-methylperhydrofuro[2,3-d]thiazole.

An extensive literature exists on the radiation-induced addition of hydrogen sulfide to olefins,[1,2] and the radiolysis of organic disulfides[3-6] in dilute solutions, in which the damaging process arises from the attack by reactive species of solvent radiolysis. The rupture of a disulfide linkage in radiation-induced reaction has been successfully correlated with the selective attack of hydrogen atoms and solvated electrons. Only a few reaction of thiamine with organic radicals, i.e. alkylperthiyl or hydroxymethyl radicals, are known. During the course of the radiolysis study of thiamine 1-adamantyl trisulfide,[7] a new radiation-induced reaction was found. This paper provides information on the products of the new radiation-induced reaction of thiamine with di-1-adamantyl tetrasulfide.

An equimolar mixture of thiamine monochloride (I) and di-1-adamantyl tetrasulfide (II) in methanol (2×10^{-3} mol/l) was irradiated with ^{60}Co gamma-rays. The thin-layer chromatographic examination of the irradiated solution revealed two products, III and IV, in addition to the unchanged I and II. The irradiated solution was chromatographed on a silica gel column, and the chemical yields of products are summarized in Table 1. The total recovery of the products and the unchanged II was 98 % based on II. The products III, mp. 198 °C, was identical in every respect with that of an authentic 3-(4-amino-2-methyl-5-pyrimidinylmethyl)-6a-(1-adamantyldithio)-3a-methylperhydrofuro[2,3-d]thiazole obtained in

the previous paper.[7] The structure of IV was determined as follows: 2-(1-Hydroxymethyl)-3-(4-amino-2-methyl-5-pyrimidinylmethyl)-6a-(1-adamantyldithio)-3a-methylperhydrofuro[2,3-d]thiazole (IV). The NMR spectrum of IV in a mixture of $CDCl_3$ and CD_3OD showed the characteristic absorption of the adamantane protons at δ=1.65, 1.95 and 2.05 which contained 6,6 and 3 protons respectively. The absorptions at δ=1.58 (s, 3H), 2.44 (s, 3H) and 7.90 (s, 1H) indicated the presence of the 3a-methyl of thiazole, pyrimidine methyl and the protons at 6-position in the pyrimidine nucleus, respectively.

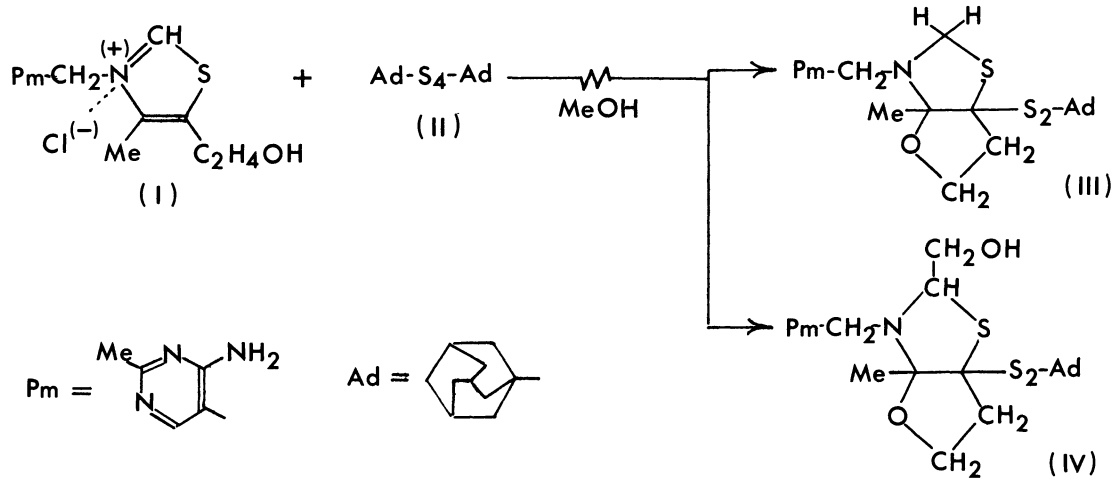
Table 1. The chemical yields of reaction products

Raw materials	mmole	Product fractions	Yield(*) (mmole)	Rf-value(**) on TLC
Thiamine monochloride (I)	6	III	0.7	0.82
		IV	2.7	0.65
Di-1-adamantyl tetrasulfide (II)	6	The unchanged II	4.2	0.96

(*) Dose, 5.94×10^{22} eV/l (dose rate, 2.37×10^{22} eV/l.h.).

(**) Silica gel precoated TLC (Tokyokasei, Ltd.) in Hexane-AcOEt-MeOH (3:3:1, v/v).

The signals were checked by the spin decoupling method and attributed to the β-protons [2.24 (m, 1H) and 2.88 (m, 1H)] and α-protons in perhydrofuro-ring [3.90 (m, 1H) and 4.18 (m, 1H)], 5-pyrimidinylmethyl [4.02 (d, 2H)], 2-hydroxymethyl of thiazole [3.66 (dd, 2H)] and the proton at 2 in thiazole [4.36 (m, 1H)]. The structure of IV as $C_{23}H_{34}O_2N_4S_3$ was further confirmed on the basis of the elemental analysis, and UV, IR and mass spectra (molecular ion, m/e=494).



Treatment of IV with anhydrous MeOH-HCl resulted in the formation of 2-hydroxymethylthiamine chloride hydrochloride, and the identity of product was confirmed by its elemental analysis, and IR, UV and NMR spectra. NMR (in D_2O, δ_{ppm}): 2.45 (s, 3H: Me-thiazole), 2.63 (s, 3H: Me-pyrimidine), 3.21 (t, 2H: βH-hydroxyethyl), 3.95 (t, 2H: αH-hydroxyethyl), 5.10 (s, 2H: 2-hydroxymethyl), 5.50 (s, 2H: 5-pyrimidinylmethyl) and 7.40 (s, 1H: H at 6 in pyrimidine).

The yield-dose curve of III and IV in the radiation-induced reaction of I with II in an argon saturated methanolic solution was measured by means of the thin-layer chromatography using Model CS-900 TLC-scanner equipped with dual wavelength (Shimadzu, Ltd.), then determined by comparison with a standard calibration curve and is shown in Fig. 1.

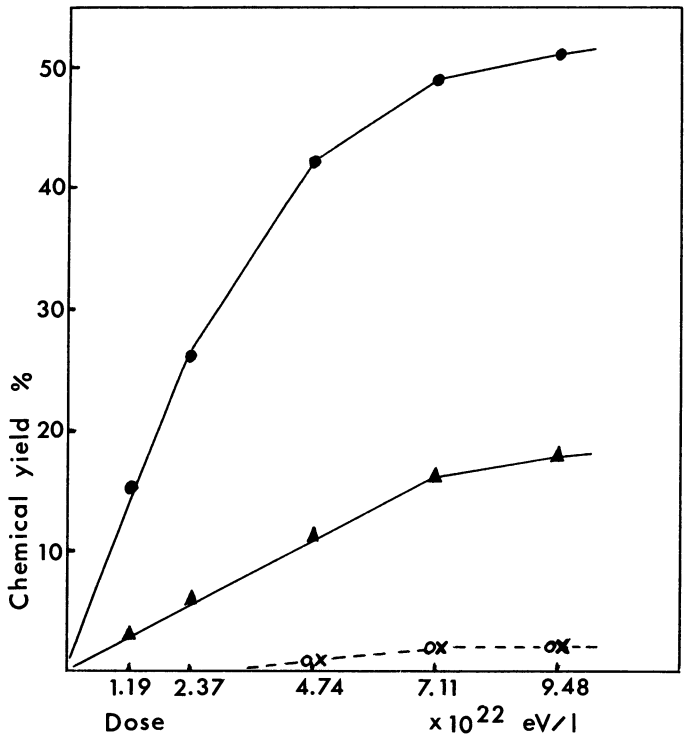


Fig. 1. The yield-dose curve of III and IV.
The reaction of II with I; yield of III (—▲—), yield of IV (—●—). The reaction of II with free-base of thiamine; yields of III and IV (-○---×--).

The chemical yields of III and IV with a dose of 9.48×10^{22} eV/l at a dose rate of 2.37×10^{22} eV/l.h. were 18% and 51%, respectively. Both G(III)=0.3 and

G(IV)=1.5 with a dose of 1.19 x 10^{22} eV/l were decreased with increasing dose. The radiation-induced reaction of II with the free-base (yellow thiol-form)[8] of thiamine which was freshly prepared by the neutralization of thiamine monochloride in methanolic-KOH, gave III (2% yield) and IV (2% yield) in low yields with a dose of 9.48 x 10^{22} eV/l.

Biological activities of III and IV

By the curative assay using thiamine-deficient rats, the growth-promoting activities of III and IV showed ca. 1/25 and 1/50 respectively based on a standard reference of thiamine.[9] By the microbiological assay using thiamine-requiring Lactobacillus viridescens (IFO-3949), thiamine activities of III and IV were also determined to be 30% and 7% respectively based on an authentic thiamine hydrochloride.

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REFERENCES

1) B.G.Dzantiyev and A.V.Shishkov, Khim. Vysok. Energii, 1, 111 (1967).
2) B.Sugimoto, W.Ando and S.Oae, Bull. Chem. Soc. Jpn., 37, 365 (1964).
3) J.W.Purdie, Can. J. Chem., 47, 1029 (1969).
4) M.Simic and M.Z.Hoffman, J. Am. Chem. Soc., 92, 6096 (1970).
5) L.K.Mee, G.Navon and G.Stein, Biochem. Biophys. Acta, 104, 151 (1965).
6) R.Shapira and G.Stein, Science, 162, 1489 (1968).
7) N.Hayashi and S.Kato, Submitted for publication. J. Labelled Compds. and Radiopharm.
8) G.D.Maier and D.E.Metzler, J.Am. Chem. Soc., 79, 4386 (1957).
9) J.Suzuoki, K.Furuno, N.Hayashi, S.Kato and T.Toga, Vitamin (Jpn), 51, 257 (1977).

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