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N-Demethylation of Dibenz[*b*,*f*]azonines with Mercuric Acetate

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The 5,6,12,13-tetrahydro-7-methyl-7*H*-dibenz[*b*,*f*]azonines (**1**), (**10**) and (**11**) were heated in the presence of mercuric acetate to yield the corresponding N-demethylated derivatives (**8**), (**13**) and (**14**), respectively.

Keywords——N-demethylation; dibenz[*b*,*f*]azonine derivative; ring expansion; benzyne reaction; isoquinoline derivative

We have previously reported a novel ring enlargement of 1-halogenobenzylisoquinolines leading to formation of N-methyl-7*H*-dibenz[*b*,*f*]azonines[2,3,4] possessing a (methylsulfinyl)-methyl group at the 13-position through the benzyne reaction using dimsylsodium[5] as a base (Chart 1).

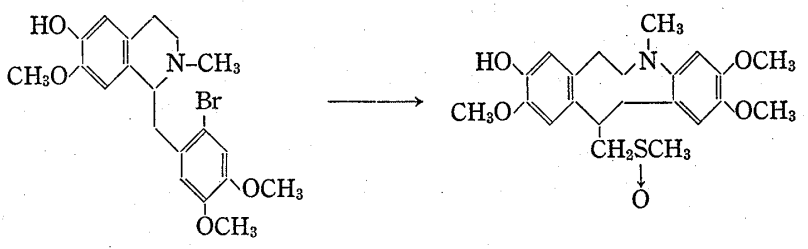

Chart 1

Since this ring enlargement was found to occur only in the case of N-methyl derivatives,[6] successively we investigated N-demethylation of N-methyl-7*H*-dibenz[*b*,*f*]azonines in order to obtain a useful intermediate leading to dibenz[*b*,*f*]azonines possessing an optional substituent at the 7-position and found that N-demethylation of N-methyl-7*H*-dibenz[*b*,*f*]azonines proceeded by treatment with mercuric acetate. We wish to report these results in this paper.

First, the 13-(methylthio)methyldibenz[*b*,*f*]azonine (**1**) was prepared from 1-(2′-bromo-4′,5′-methylenedioxybenzyl)-1,2,3,4-tetrahydro-6-hydroxy-7-methoxy-2-methylisoquinoline (**2**) by the same manner as that we reported previously.[2] The isoquinoline (**2**) was obtained from the amine (**3**) through the general method (**3**→**4**→**5**→**6**→**2**) as described in the experimental section (see Chart 2). Treatment of **2** with dimsylsodium in dimethyl sulfoxide afforded the 13-(methylsulfinyl)methyl derivative (**7**), which was deoxygenated with zinc amalgam to give **1**.

Secondly, the 13-(methylthio)methyldibenz[*b*,*f*]azonine (**1**) was heated with an equimolar amount of mercuric acetate in 30% acetic acid in the expectation that the N-demethylated dibenz[*b*,*f*]azonine (**8**) would be formed through the iminium salt (**9**) as in the case of some

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2) S. Kano, E. Komiyama, T. Ogawa, Y. Takahagi, T. Yokomatsu, and S. Shibuya, *Chem. Pharm. Bull.* (Tokyo), **23**, 2058 (1975).
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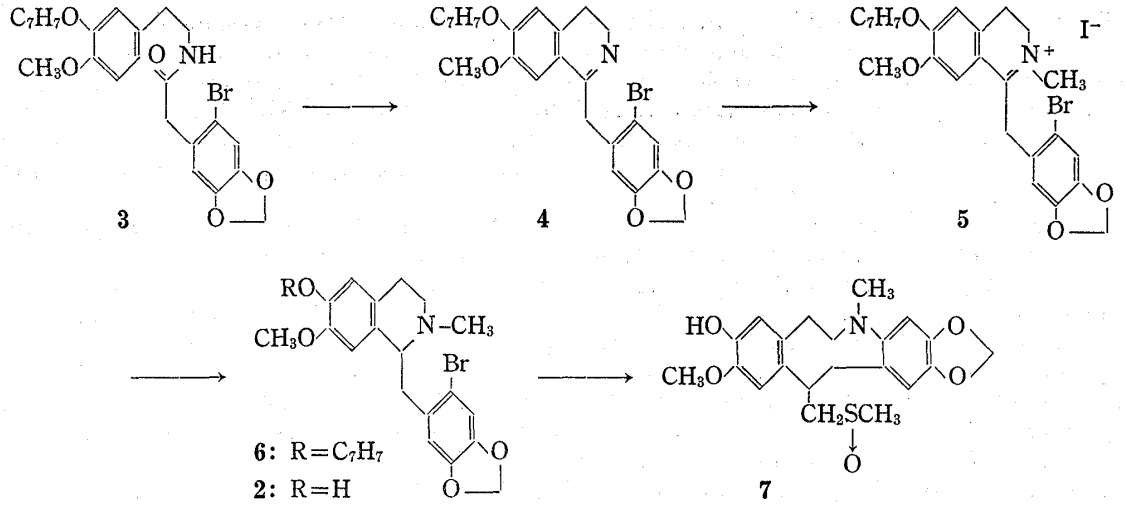
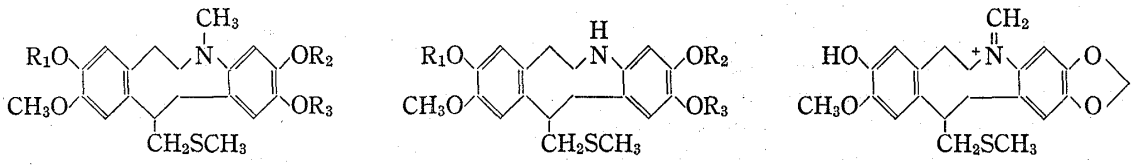


Chart 2

bicyclic amines.[7,8] The molecular formula of the product was determined as $C_{20}H_{23}NO_4S$ by microanalysis and mass spectrum (M^+, *m/e* 373). Its nuclear magnetic resonance (NMR) ($CDCl_3$) spectrum exhibited no signal assigned to NCH_3. The signal due to SCH_3 appeared at 1.98 ppm. The Eschweiler-Clarke reaction of **8** with 99% formic acid and 37% formalin afforded **1**. On the basis of these results, the structure of the product from **1** was confirmed to be **8**. However, the same treatment of **7** with mercuric acetate resulted in failure. It is of interest to investigate the same reaction using the non-phenolic derivatives (**10**) and (**11**) in order to examine whether any difference was observed in this reaction between the phenolic and non-phenolic dibenz[*b*,*f*]azonines. The non-phenolic derivatives (**10**) and (**11**) were obtained from **1** and **12**,[2] respectively, by O-methylation with diazomethane in methanol. Treatment of **10** and **11** with mercuric acetate gave the corresponding N-demethylated products (**13**) and (**14**), respectively, as in the case of **1**. Thus, it was found that a methyl group of N-methyl dibenz[*b*,*f*]azonines was removed by treatment with mercuric acetate.



1: R_1=H, R_2-R_3=-CH_2-
10: R_1=CH_3, R_2-R_3=-CH_2-
11: R_1=R_2=R_3=CH_3
12: R_1=H, R_2=R_3=CH_3

8: R_1=H, R_2-R_3=-CH_2-
13: R_1=CH_3, R_2-R_3=-CH_2-
14: R_1=R_2=R_3=CH_3

9

Chart 3

Experimental[9]

N-(3-Benzyloxy-4-methoxyphenethyl)-2-bromo-4,5-methylenedioxyphenylacetamide (3)——A mixture of 6 g of 3-benzyloxy-4-methoxyphenethylamine and 6 g of 2-bromo-4,5-methylenedioxyphenylacetic acid was heated at 180° for 1.5 hr. After cooling, the mixture was recrystallized from MeOH to give 11 g of **3**, mp 181—183°. *Anal.* Calcd. for $C_{25}H_{24}O_5NBr$: C, 60.25; H, 4.85; N, 2.81. Found: C, 60.70; H, 4.66; N, 2.79.

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8) F. Bohlmann and P. Strehlke, *Tetrahedron Letters*, **1965**, 167.
9) All melting points were uncorrected. NMR spectra were taken with a Varian T-60 spectrometer in $CDCl_3$ using TMS as an internal standard unless otherwise noted. Mass spectra were measured with a Hitachi RMU-7L spectrometer.

6-Benzyloxy-1-(2′-bromo-4′,5′-methylenedioxybenzyl)-3,4-dihydro-7-methoxyisoquinoline (4)——A mixture of 10 g of **3**, 10 g of $POCl_3$ and 200 ml of benzene was refluxed for 1.5 hr. To the reaction mixture was added excess *n*-hexane and the whole was allowed to stand for 10 hr and the supernatant liquid was decanted. The precipitate was made basic with 28% NH_4OH and extracted with $CHCl_3$. The extract was washed with H_2O, dried (Na_2SO_4) and evaporated. The resulting solid was recrystallized from MeOH–ether to give 8.5 g of **4** as colorless needles, mp 125—127°. *Anal.* Calcd. for $C_{25}H_{22}O_4NBr$: C, 62.51; H, 4.62; N, 2.92. Found: C, 62.78; H, 4.39; N, 2.86.

6-Benzyloxy-1-(2′-bromo-4′,5′-methylenedioxybenzyl)-1,2,3,4-tetrahydro-7-methoxy-2-methylisoquinoline (6)——A mixture of 8 g of **4**, 20 ml of CH_3I and 150 ml of MeOH was refluxed for 4 hr and the solvent was evaporated. To a methanolic solution of the resulting methiodide (**5**) Tas added 4 g of $NaBH_4$ under stirring at room temperature within 0.5 hr, and the mixture was refluxed for 1 hr. The remaining residue, obtained after evaporation of the solvent, was suspended in H_2O and extracted with $CHCl_3$. The extract was washed with H_2O, dried over Na_2SO_4, and evaporated to yield 7.5 g of **6** as colorless needles, mp 101—103° (MeOH–ether). *Anal.* Calcd. for $C_{26}H_{26}O_4NBr$: C, 62.91; H, 5.28; N, 2.82. Found: C, 63.16; H, 5.28; N, 2.77.

1-(2′-Bromo-4′,5′-methylenedioxybenzyl)-1,2,3,4-tetrahydro-6-hydroxy-7-methoxy-2-methylisoquinoline (2)——A mixture of 5 g of **6**, 30 ml of conc. HCl and 50 ml of EtOH was refluxed for 2 hr. The solvent was removed and the remaining residue was made basic with 28% NH_4OH and extracted with $CHCl_3$. The extract was washed with H_2O, dried (Na_2SO_4) and evaporated. The remaining residue was recrystallized from MeOH–ether, mp 143—144°. NMR ($CDCl_3$) δ: 2.48 (3H, singlet, NCH_3), 3.62 (3H, singlet, OCH_3), 5.85 (2H, singlet, OCH_2O), 6.08 (1H, singlet, 8-H), 6.55 (2H, singlet, 5-H and 6′-H), 6.94 (1H, singlet, 3′-H). *Anal.* Calcd. for $C_{19}H_{20}O_4NBr$: C, 56.17; H, 4.96; N, 3.45. Found: C, 56.52; H, 4.86; N, 3.25.

5,6,12,13-Tetrahydro-3-hydroxy-2-methoxy-7-methyl-9,10-methylenedioxy-13-(methylsulfinyl)methyl-7*H*-dibenz[*b*,*f*]azonine (7)——To a solution of dimsylsodium (prepared from 4 g of NaH and 30 ml of dimethyl sulfoxide (DMSO) was added a solution of 3.5 g of **2** in 35 ml of DMSO at room temperature under stirring. After the stirring had been continued for 14 hr, the mixture was poured into 300 ml of H_2O containing excess NH_4Cl and extracted with $CHCl_3$. The extract was washed with H_2O, dried over Na_2SO_4 and evaporated. The resulting solid was recrystallized from MeOH–ether to yield 3.1 g of **7** as colorless needles, mp 206—209°. Mass Spectrum *m/e*: 403 (M^+). *Anal.* Calcd. for $C_{21}H_{25}O_5NS$: C, 62.51; H, 6.25; N, 3.47. Found: C, 62.28; H, 6.33; N, 3.24.

5,6,12,13-Tetrahydro-3-hydroxy-2-methoxy-7-methyl-9,10-methylenedioxy-13-(methylthio)methyl-7*H*-dibenz[*b*,*f*]azonine (1)——A mixture of 2 g of **7**, 30 ml of conc. HCl, 30 ml of 50% AcOH, and Zn–Hg (prepared from 1 g of $HgCl_2$ and 10 g of Zn) was heated on a water bath for 2 hr. After removal of inorganic substance, the mixture was made basic with 28% NH_4OH and extracted with $CHCl_3$. The extract was washed with H_2O, dried (Na_2SO_4) and evaporated to leave 1.7 g of **1** as colorless needles, mp 174—174.5° (MeOH–ether). NMR ($CDCl_3$) δ: 2.13 (3H, singlet, SCH_3), 2.55 (3H, singlet, NCH_3), 3.85 (3H, singlet, OCH_3), 5.90 (2H, singlet, OCH_2O), 6.63, 6.70, 6.78, 6.88 (4H, each singlet, $4\times$Ar–H). *Anal.* Calcd. for $C_{21}H_{25}O_4NS$: C, 65.09; H, 6.50; N, 3.62. Found: C, 64.99; H, 6.80; N, 3.32.

5,6,12,13-Tetrahydro-2,3-dimethoxy-7-methyl-9,10-methylenedioxy-13-(methylthio)methyl-7*H*-dibenz[*b*,*f*]azonine (10)——To a solution of 1.5 g of **1** in 50 ml of MeOH was added a solution of excess diazomethane in ether and the mixture was allowed to stand for 10 hr. Removal of the solvent left 1.5 g of **10** as colorless needles, mp 108—109° (ether). Mass Spectrum *m/e*: 401 (M^+). NMR ($CDCl_3$) δ: 2.07 (3H, singlet, SCH_3), 2.50 (3H, singlet, NCH_3), 3.80, 3.82 (6H, each singlet, $2\times OCH_3$), 5.82 (2H, singlet, OCH_2O), 6.55, 6.58, 6.68, 6.77 (4H, each singlet, $4\times$Ar–H). *Anal.* Calcd. for $C_{22}H_{27}O_4NS$: C, 65.81; H, 6.78; N, 3.49. Found: 65.52; H, 6.91; N, 3.33.

5,6,12,13-Tetrahydro-2,3,9,10-tetramethoxy-7-methyl-13-(methylthio)methyl-7*H*-dibenz[*b*,*f*]azonine (11)——To a solution of 1.5 g of **12**[2] in 50 ml of MeOH was added a solution of excess diazomethane in ether and the mixture was worked up as above to yield 1.5 g of **11** as colorless needles, mp 105—107° (ether). NMR ($CDCl_3$) δ: 2.08 (3H, singlet, SCH_3), 2.53 (3H, singlet, NCH_3), 3.83 (12H, singlet, $4\times OCH_3$), 6.60, 6.58, 6.73, 6.87 (4H, each singlet, $4\times$Ar–H). *Anal.* Calcd. for $C_{23}H_{31}O_3NS$: C, 66.16; H, 7.48; N, 3.36. Found: C, 66.02; H, 7.64; N, 3.29.

5,6,12,13-Tetrahydro-3-hydroxy-2-methoxy-9,10-methylenedioxy-13-(methylthio)methyl-7*H*-dibenz[*b*,*f*]azonine (8)——A mixture of 1.5 g of **1**, 1 g of $Hg(OAc)_2$ and 120 ml of 30% AcOH was heated on a water bath for 2 hr. The mixture was made basic with 28% NH_4OH and extracted with $CHCl_3$. The extract was washed with H_2O, dried over Na_2SO_4, and evaporated. The remaining residue was chromatographed on 10 g of silica gel using $CHCl_3$ as an eluant. Removal of the solvent (70 ml) afforded 0.3 g of **8** as colorless needles, mp 182—183° (MeOH–ether). Mass Spectrum *m/e*: 373 (M^+). NMR ($CDCl_3$) δ: 1.98 (3H, singlet, SCH_3), 3.77 (3H, singlet, OCH_3), 5.80 (2H, singlet, OCH_2O), 6.50 (1H, singlet, Ar–H), 6.62 (2H, singlet, $2\times$Ar–H), 6.68 (1H, singlet, Ar–H). *Anal.* Calcd. for $C_{20}H_{23}O_4NS$: C, 64.32; H, 6.21; N, 3.75. Found: C, 64.04; H, 3.50; N, 6.24.

5,6,12,13-Tetrahydro-2,3-dimethoxy-9,10-methylenedioxy-13-(methylthio)methyl-7*H*-dibenz[*b*,*f*]azonine (13)——A mixture of 1 g of **10**, 0.8 g of $Hg(OAc)_2$ and 100 ml of 30% AcOH was heated on a water bath for 2 hr and the mixture was worked up as above to give 0.2 g of **13** as an oil. Mass Spectrum *m/e*: 387 (M^+). NMR ($CDCl_3$) δ: 2.00 (3H, singlet, SCH_3), 3.87 (6H, singlet, $2\times OCH_3$), 5.83 (2H, singlet, OCH_2O), 6.50

(1H, singlet, Ar–H), 6.60 (1H, singlet, Ar–H), 6.68 (2H, singlet, 2×Ar–H). Styphnate, mp 128—130° (MeOH–ether). *Anal.* Calcd. for $C_{27}H_{28}O_{12}N_4S$: C, 51.26; H, 4.46; N, 8.85. Found: C, 51.26; H, 4.52; N, 8.65.

5,6,12,13-Tetrahydro-2,3,9,10-tetramethoxy-13-(methylthio)methyl-7*H*-dibenz[*b*,*f*]azonine (14)——A mixture of 1.1 g of **12**, 0.8 g of $Hg(OAc)_2$ and 100 ml of 30% AcOH was heated in a water bath for 2 hr. The mixture was worked up as above to give 0.2 g of **14**, mp 148—150° (MeOH–ether). Mass Spectrum *m/e*: 403 (M^+). NMR ($CDCl_3$) δ: 2.00 (3H, singlet, SCH_3), 6.55, 6.60, 6.70, 6.78 (4H, each singlet, 4×Ar–H). *Anal.* Calcd. for $C_{22}H_{29}O_4NS$: C, 65.48; H, 7.24; N, 3.47. Found: C, 65.61; H, 7.38; N, 3.47.

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Purification and Some Properties of Violet-colored Acid Phosphatase from Spinach Leaves

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The violet-colored acid phosphatase was purified from leaves of spinach (*Spinacia oleracea*). The purified preparation was found to be homogeneous by electrophoresis and ultracentrifugation. Concentrated solution of the enzyme had an intense violet color with a broad absorption band between 410 nm and 700 nm. The peak was at around 530 nm. The molecular weight of the enzyme was approximately 92000. The enzyme was composed of two subunits of equal size and contained manganese. The result of staining of the enzyme in disc electrophoresis gel by periodic acid–Schiff reagent indicated that the enzyme was glycoprotein.

Keywords——acid phosphatase; violet-colored acid phosphatase; spinach; phosphatase purification; manganese; *Spinacia oleracea*

Acid phosphatases have been isolated in various states of purity from many different living organisms, and there have been numerous reports dealing with the properties of these enzymes.[2] Violet-colored acid phosphatase containing manganese have been found recently in sweet potato[3] and rice plant cultured cells.[4] However, the substantial nature of the color, manganese requirement and subcellular distribution of the enzyme are still unknown.

During the course of our investigations of the colored acid phosphatase, it was found that leaves of spinach (*Spinacia oleracea*) also contained a violet-colored acid phosphatase containing manganese. In this communication we report the results of purification and some properties of this enzyme.

Material and Method

Materials——Spinach leaves were obtained from a local market.

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