ARTICLES

Identification and Synthesis of New Odor Compounds from Photolysis of Thiamin

Hans M. van Dort,* Leendert M. van der Linde, and David de Rijke

The identity of two new aroma compounds 1-methylbicyclo[3.3.0]-4-thia-2,8-dioxaoctane and 1,3-dimethylbicyclo[3.3.0]-4-thia-2,8-dioxaoctane, related to the characteristic odor of thiamin preparations, is reported, and their synthesis is described. These compounds are formed by photolysis (2537 Å) of thiamin hydrochloride at 35 °C along with two other new compounds: 2-methyl-3-formyl-4,5-dihydrofuran and 3-acetyl-4,5-dihydrofuran. Spectral data, odor thresholds, and odor descriptions are given. A possible pathway for the formation of these compounds from thiamin is proposed.

In 1978 Seifert et al. reported the isolation of 1-methylbicyclo[3.3.0]-8-oxa-2,4-dithiaoctane (4) from UV-irradiated thiamin. The synthesis of 4 was reported by Gygax (1981). Initially 4 was believed to be responsible for the typical odor of "thiamin preparations" (Buttery et al., 1981); however, a difference in odor threshold between the isolated and synthesized compounds (4 × 10⁻¹³ and ±10⁻⁹, respectively) has led to reconsideration.

Another compound, unidentified, was suggested to be even more important than 4 (Buttery and Seifert, 1982). This ambiguity prompted us to report the identification and synthesis of several new compounds from irradiated thiamin. None of these compounds, however, had an odor threshold significantly lower than that of 4. It is therefore unlikely that one of these compounds is the unknown component found by Buttery and Seifert (1982). Two of the products formed upon photolysis of thiamin are closely related to 4.

EXPERIMENTAL SECTION

Mass spectra were performed on a Varianmat CH 7A spectrometer. ¹H NMR spectra were obtained on a 100-Mz Jeol-type FX-100 spectrometer. Samples were run in carbon tetrachloride, with tetramethylsilane as an internal reference. Infrared spectra of thin liquid films were obtained on a Perkin-Elmer Model 457 (measurements in cm⁻¹) and Model 137 E (measurements in μm).

Photolysis of Thiamin Hydrochloride. A solution of 177 g of food-grade thiamin hydrochloride (Hoffmann-La Roche) in 750 mL of demineralized water was prepared in a 1-L quartz flask equipped with an internal cooling jacket, nitrogen inlet, and condenser. The solution was irradiated for 5 days (120 h) in a Rayonet photochemical reactor (RPR 2088 the Southern New England Ultraviolet Co.) equipped with a circular group of eight 2537-Å U-form Rayonet lamps (total input 80 W). The temperature of the reaction mixture during irradiation was 35 °C. After saturation with salt, the flask contents were extracted continuously with ether for 24 h. After the extract was dried with magnesium sulfate, the ether was stripped off in a rotavapor (<30 °C, 50 mmHg) and the residue distilled: 0.2 g of oily distillate was obtained (bp₃ 50 °C),

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Scheme I
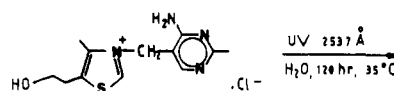
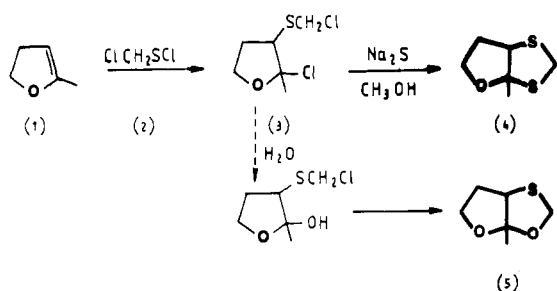| COMPOUND	FORMULA	%	NR.
1-methyl bicyclo[3.3.0]-2,8-dioxa-4-thia octane		~ 4	5
1,3-dimethyl bicyclo[3.3.0]-2,8-dioxa-4-thia octane		29,2	6
2-methyl-3-formyl-4,5-dihydrofuran		18,0	7
3-acetyl-4,5-dihydrofuran		17,6	8
5-formyloxy-2-pentanon	HC-O-CH₂-CH₂-CH₂-C-CH₃	7,7	9
3-formyl-5-hydroxy-2-pentanon	HO-CH₂CH₂-CH-C-CH₃	4,6	10
2-methyl-3-mercapto-4,5-dihydrofuran		5,6	11
bis(2-methyl-4,5-dihydrofuran-3-yl)disulfide		~ 9	12
unidentified minor compounds		~ 5	

which represents a yield of about 0.1% w/w.

Purification and Analysis. The distillate was purified by repeated preparative GLC at 160 °C on a 2 m × ¹/₄ in. glass column, packed with 60-80-mesh Chromosorb G, coated with 10% DEGS. Injector, detector, and outlet system temperatures were 200 °C; the hydrogen carrier gas flow was 60 mL/min.

Four peaks were collected separately; MS/NMR/IR analysis indicated the presence of at least eight components (Scheme I).

Synthesis of Compounds. *2-Methyl-4,5-dihydrofuran* (1). This compound was synthesized as described in Houben-Weyl (1965) from α-acetyl-γ-butyrolactone in 40% yield: bp₁₆ 100 °C; IR (liquid capillary) 3.20, 5.95, 6.75,

Scheme II

SCH₂Cl / ClCH₂SCl → ... Cl / Na₂S, CH₃OH → (4)

(1) (2) (3) | H₂O ↓ SCH₂Cl ... OH → (5)

Scheme III

$C-C-O-C-C-C-C-C$ $\xrightarrow{H_2S, N(C_2H_5)_3}$ $C-C-O-C-C-C-C$ (SH)

(13) (14)

$(14) + C-C-O-CH_2Br$ $\xrightarrow[(2) H^+/\Delta T, CH_3OH]{(1) N(C_2H_5)_3}$ (5)

(15)

$(14) + C-C-O-C-C$ (Cl) $\xrightarrow[(2) H^+ \Delta T, CH_3OH]{(1) N(C_2H_5)_3}$ (6)

(16)

6.90, 7.25, 7.30, 8.05, 8.45, 9.85, 9.95, 10.40, 10.80, 11.15, 13.85 μm; NMR (CCl₄, 100 MHz) δ 1.72 (3 H, t of d; J_t = 2 Hz, J_d = 1.2 Hz), 2.55 (2, H, m), 4.23 (2 H, t, J = 9 Hz), 4.43, (1 H, m); mass spectrum 27 (19), 29 (9), 39 (34), 41 (22), 43 (100), 53 (14), 55 (16), 69 (9), 83 (27), 84 (41).

(Chloromethane)sulfenyl Chloride (2). This compound was prepared by repeated chlorination of dimethyl disulfide with sulfuryl chloride. The product could not be obtained in the pure state (assay ≤50%) (Brintzinger et al., 1950, 1952; Douglass et al., 1951): NMR (CCl₄, 100 MHz) δ 5.10 (s).

1-Methylbicyclo[3.3.0]-2,4-dithia-8-oxaoctane **(4):** *Synthesis according to Scheme II.* A solution of 6 g (maximum of 0.05 mol) of (chloromethyl)sulfenyl chloride (2) in 70 mL of carbon tetrachloride was cooled to 0–10 °C, and a solution of 10 g (0.12 mol) of 2-methyl-4,5-dihydrofuran (1) in 20 mL of carbon tetrachloride was added dropwise with cooling and stirring. An exothermic reaction took place (→35 °C).

The reaction mixture was then stirred for 1 h at room temperature and washed twice with cold water. The resulting neutral carbon tetrachloride solution was dried on magnesium sulfate.

The crude 2-chloro-2-methyl-3-[(chloromethyl)thio]-tetrahydrofuran (3) so obtained was not purified but converted directly to 4 by adding it dropwise, with cooling, to a well-stirred solution of 80 g (0.2 mol) of sodium sulfide in methanol. An exothermic reaction occurred, and the temperature rose to 30 °C.

After subsidence of the exothermic reaction, the reaction mixture was stirred for 2 h at room temperature and poured onto ice. The alkaline reaction mixture was neutralized with diluted hydrochloric acid and extracted twice with methylene chloride. The combined extracts were washed with water, dried on magnesium sulfate, and concentrated. The residue obtained was distilled in a small distillation apparatus. The distillate (bp₃ ±50–125 °C, 0.5 g) consisted mainly of two compounds that were purified by preparative GLC as described above.

The first compound was the title compound (4); spectral data were identical with those reported by Seifert et al. (1978). The second compound proved to be 1-methyl-bicyclo[3.3.0]-thia-2,8-dioxaoctane (5), an oxygen analogue. The structure was confirmed by a more direct synthesis.

1-Methylbicyclo[3.3.0]-4-thia-2,8-dioxaoctane **(5).** This new compound was synthesized according to Scheme III. Reaction of bromomethyl acetate (15) (Uhlich and Adams 1921) with 5-acetoxy-3-mercapto-2-pentanone (14) (Gygax, 1981) and triethylamine, followed by acid-catalyzed ring closure, yielded the title compound. Spectral data were identical with those of the second component described before and the isolated component from thiamin irradiation: IR (CCl₄ + CS₂) 3985, 2937, 2879, 1481, 1380, 1355, 1299, 1281, 1199, 1163, 1144, 1109, 1055, 1031, 1005, 985, 939, 902, 854, 807, 733, 720, 609, 587, cm⁻¹; NMR (CCl₄, 100 MHz) δ 1.52 (3 H, s), 1.9–2.6 (2 H, m), 3.60 (1 H, d),
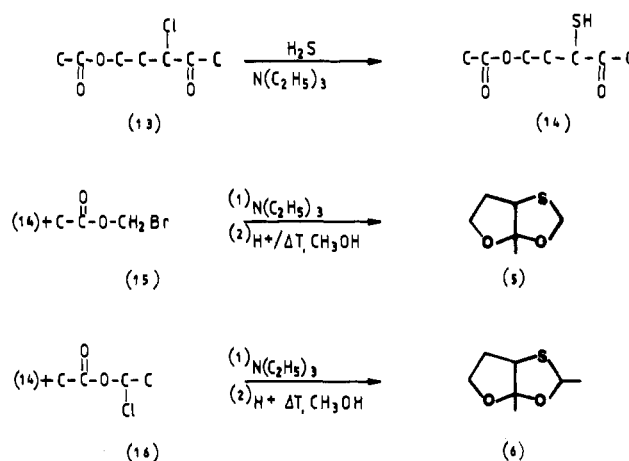
3.8–4.2 (2 H, m), 4.76 (1 H, d, J = 7 Hz), 4.89 (1 H, d, J = 7 Hz); mass spectrum (rel intensity) 39 (13), 41 (9), 43 (100), 45 (25), 55 (7), 71 (13), 73 (24), 83 (25), 85 (27), 86 (39), 88 (9), 101 (3), 116 (52), 146 (25).

1,3-Dimethylbicyclo[3.3.0]-4-thia-2,8-dioxaoctane **(6).** This compound was also prepared according to scheme III. Reaction of 1-chloroethyl acetate (16) [prepared from acetyl chloride and acetaldehyde; bp₇₅₀ 120 °C; yield 30%; method of Uhlich and Adams (1921)] with 5-acetoxy-3-mercapto-2-pentanone (14) and triethylamine, followed by acid-catalyzed ring closure, yielded the title compound. Spectral data were identical with those of the isolated component from thiamin irradiation (two isomers): IR (CCl₄ + CS₂) 2988, 2951, 2933, 2880, 1475, 1448, 1441, 1381, 1378, 1354, 1308, 1279, 1263, 1175, 1149, 1124, 1076, 1040, 1024, 1004, 962, 936, 902, 876, 854, 812, 668, 635, 582, cm⁻¹; NMR (CCl₄, 100 MHz) δ (a) 1.50 (3 H, d, J = 6 Hz), 1.5 (3 H, s), 1.7–2.6 (2 H, m), 3.75 (1 H, d of d), 3.6–4.3 (2 H, m), 5.33 (1 H, q, J = 6 Hz); (b) 1.50 (3 H, s), 1.55 (3 H, d, J = 6 Hz), 1.7–2.6 (2 H, m), 3.62 (1 H, d of d), 3.6–4.3 (2 H, m), 5.03 (1 H, q, J = 6 Hz); a and b are stereoisomers; mass spectrum (rel intensity) 160 (M⁺; 1), 117 (11), 116 (95), 85 (12), 84 (12), 83 (27), 74 (8), 73 (21), 60 (8), 45 (13), 43 (100).

2-Methyl-3-formyl-4,5-dihydrofuran **(7).** This compound was not synthesized. Spectral data of the isolated product are as follows: IR (CCl₄/CS₂) 2975, 2930, 2906, 2875, 2822, 2761, 2713, 1659, 1644, 1478, 1453, 1401, 1391, 1369, 1337, 1236, 1229, 988, 971, 919, 616 cm⁻¹; NMR (CCl₄, 100 MZh) δ 2.18 (3 H, t), 2.78 (2 H, t, J = 9 Hz), 4.48 (2 H, t, J = 9 Hz), 9.64 (1 H, s); mass spectrum (rel intensity) 39 (24), 41 (20), 43 (100), 53 (13), 69 (65), 83 (15), 97 (18), 111 (42), 112 (98).

3-Acetyl-4,5-dihydrofuran **(8).** This compound was not synthesized. Spectral data of the isolated product are as follows: IR (CCl₄/CS₂) 3093, 2971, 2928, 2903, 2875, 1659, 1612, 1476, 1450, 1377, 1338, 1134, 983, 943, 894, 866, 601, 456 cm⁻¹; NMR (CCl₄, 100 MHz) δ 2.16 (3 H, s), 2.76 (2 H, t of d, J = 9 Hz), 4.51 (2 H, t), 7.24 (1 H, t); mass spectrum (rel intensity) 39 (18), 41 (52), 43 (69), 53 (4), 69 (43), 84 (4), 97 (100), 112 (96).

5-(Formyloxy)-2-pentanone **(9).** This compound was not synthesized. Spectral data of the isolated product are as follows: IR (CCl₄/CS₂) 1732, 1725 (sh), 1412, 1369, 1357, 1171 (sh), 1164 cm⁻¹; NMR (CCl₄, 100 MHz) δ 1.87 (2 H, q, J = 7 Hz), 2.11 (3 H, s), 2.53 (2 H, t, J = 7 Hz), 4.11 (2 H, t, J = 7 Hz), 7.99 (1 H, s).

2-Methyl-3-mercapto-4,5-dihydrofuran **(11).** This compound was also found as a thermal degradation product of thiamin (van der Linde et al., 1978) and de-

Scheme IV

Scheme V

Scheme VI
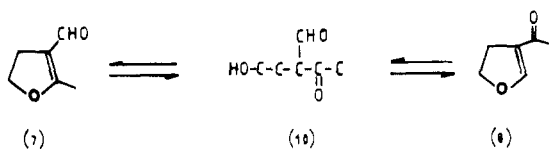
scribed by van der Ouweland and Peer (1975).

Bis(2-methyl-4,5-dihydrofuryl-3) Disulfide (**12**). This is a well-known aroma component (IFF, 1970).

RESULTS AND DISCUSSION

In the course of our work on the degradation of thiamin (van der Linde et al., 1978) we synthesized 1-methyl-bicyclo[3.3.0]-8-oxa-2,4-dithiaoctane (**4**) via a route shown in Scheme II. Addition of (chloromethyl)sulfenyl chloride (**2**) to 2-methyl-4,5-dihydrofuran (**1**) resulted in **3**. Reaction of **3** with sodium sulfide in methanol produced a mixture of **4** and **5**. The latter is an oxygen analogue of **4**: 1-methylbicyclo[3.3.0]-2,8-dioxa-4-thiaoctane. **5** can be formed from **3** by hydrolysis. This new compound has the same typical "thiamin" odor as **4**, although somewhat less powerful.
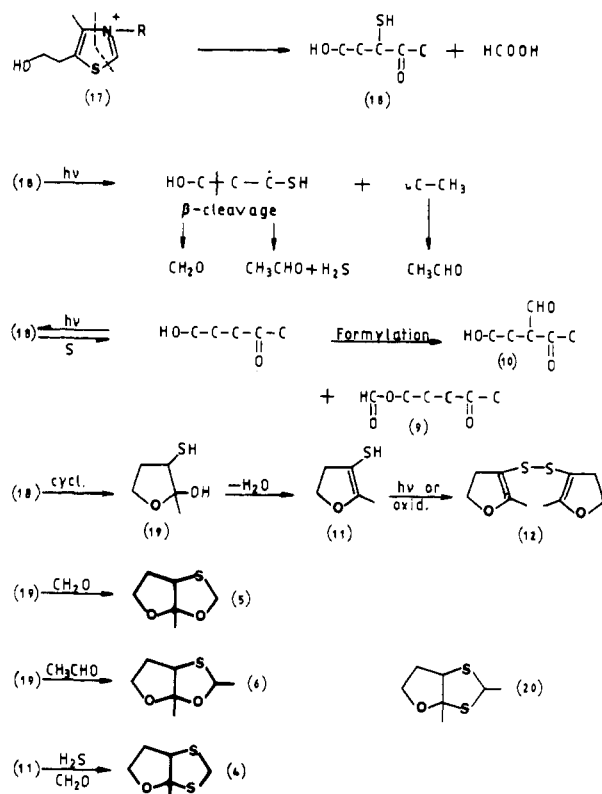
Irradiation of Thiamin. The formation of the new compound (**5**) as a byproduct of **4** prompted us to repeat the photolysis experiment of Seifert et al. (1978) to see whether or not the new compound is also formed as a photodegradation product of thiamin. The results are given in Scheme I: a number of new compounds, among which is the new aroma compound, were isolated and identified (MS, NMR, IR).

When Scheme I is studied, it is striking that the results of Seifert and Buttery are not duplicated: compound **4** was not found. Instead of **4** a compound with the formula **6**—1,3-dimethylbicyclo[3.3.0]-2,8-dioxa-4-thiaoctane— appeared to be the main component, with compound **5** as a minor component. The difference in reaction conditions (temperature, amount of radiation, etc.) might explain these differences as well as the formation of different quantities of reactive UV-degradation intermediates, e.g., hydrogen sulfide, formaldehyde, acetaldehyde, etc. The structures of the compounds **5** and **6** could be confirmed by synthesis according to Scheme III [method of Gygax (1981)]. The NMR spectra indicate that compound **6** exists as two isomers.
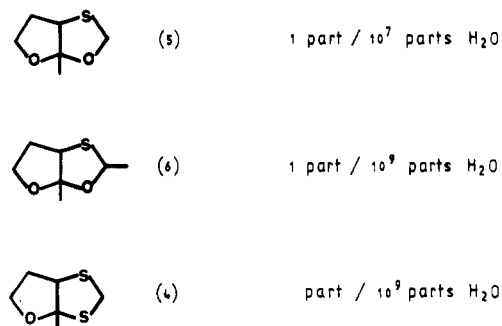
Two other new non-sulfurous compounds isolated from UV-irradiated thiamin are 2-methyl-3-formyl-4,5-di-hydrofuran (**7**) and 3-acetyl-4,5-dihydrofuran (**8**). Both compounds form an equilibrium mixture via the ring-opened intermediate (**10**) (Scheme IV). The other components found (**11** and **12**) were reported before (IFF, 1970; van der Linde et al., 1978).

Possible Reaction Mechanism. A possible reaction mechanism for the formation of the "thiamin odor compound" (**4**) is given by Buttery et al. (1981). However, this scheme does not account for the formation of the compounds we isolated from thiamin photolysis; therefore, we propose the mechanism shown in Scheme V. The first step in thiamin degradation is proposed to be the disruption of the C–N– and C–S– bonds resulting in compound **18**, which can be considered as the key intermediate in the formation of all degradation products. Formation of this compound in heated thiamin solutions is demonstrated by Matsukawa et al. (1951). The same intermediate was proposed in the thermal degradation of thiamin (van der Linde et al., 1978); thus, the first degradation step may be the result of a photochemical or a hydrolysis reaction. The next step could be the formation of hydrogen sulfide, formaldehyde, and acetaldehyde from **18** via a photo-

chemical reaction. Under the same conditions sulfur can be eliminated, resulting after formylation in the formation of **9** and **10**. On cyclization compound **10** gives an equilibrium mixture of **7** and **8** (Scheme IV). Mercaptan (**11**) is formed upon dehydration of **18**; oxidation or radical reaction gives the disulfide. These last two compounds are not typical photolysis products; they also occur in thermal breakdown. When **19** reacts with formaldehyde or acetaldehyde, the new compounds **5** and **6** are formed. At the same time **4** can be formed by H$_2$S addition to **11** followed by reaction with formaldehyde; however, this obviously does not occur under our conditions. In the same way the methyl derivative (**20**) could be formed from reaction with acetaldehyde. Therefore, compounds **5–10** only are typical final products of thiamin photolysis under our conditions.

Odor Threshold and Description. Odor thresholds of the thiamin odor compounds **4–6** were measured by a panel of experts, who smelled and tasted solutions of the compounds in water. The values determined are shown in Scheme VI. These thresholds are in agreement with the revised measurements of Buttery and Seifert (1982). The odors were described as sulfury, metallic green, somewhat like sulfurol, and sweet.

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Metabolism of Limonoids: Conversion of Nomilin to Obacunone in *Corynebacterium fascians*

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Corynebacterium fascians cells immobilized in acrylamide gel converted nomilin to obacunone in orange juice serum. Cell-free extracts of the organism also catalyzed this conversion. The conversion of nomilin to obacunone is the fourth metabolic pathway of nomilin established in bacteria.

Bitterness due to limonoids in certain citrus juices is one of the major problems of the citrus industry worldwide and has significant economic impact. During the course of our study on limonoid metabolism, we have isolated from soil a strain of Bacterium, *Corynebacterium fascians* (Hasegawa and King, 1983). Unlike other limonoid-metabolizing bacteria (Hasegawa et al., 1972a,b, 1974, 1983; Hasegawa and Kim, 1975; Vaks and Lifshitz, 1981), this bacterium produces constitutive enzymes for limonoid metabolism that is advantageous over the others from a practical viewpoint. Cells, capable of metabolizing limonoids, can be produced conveniently and economically with simple carbon sources and could be used for a biological process that uses immobilized bacterial cells for reduction of limonoid bitterness of citrus juices (Hasegawa et al., 1982a). The enzymes in the other bacteria had to be induced.

At least 29 limonoids have been isolated from *Citrus* and *Citrus* hybrids; four of them, limonin (1) (Figure 1), nomilin (5), (Dreyer, 1963) (Figure 2), ichangin (Dreyer, 1966), and nomilinic acid (Hasegawa and Bennett, 1975), are bitter. Limonin is the major limonoid present in citrus juices and the primary cause of limonoid bitterness. Recently, it was shown that nomilin also appears to play a role in limonoid bitterness (Rouseff et al., 1981; Hashinaga and Itoo, 1981). Therefore, nomilin has been included in our citrus juice debittering study.

Recently, we have found that treatment of orange juice serums with *C. fascians* immobilized in acrylamide gel

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converted nomilin to three unidentified metabolites. The major metabolites, which consisted of approximately 90% of the total metabolites, was, therefore, isolated and its structure determined.

MATERIALS AND METHODS

Valencia oranges were purchased from a local market. The juice was extracted with a Sunkist juicer. Nomilin was dissolved in a minimal portion of CH_3CN and added to the juice to bring its concentration to 20–40 ppm. The serum was obtained from the juice by centrifugation at 5000g for 30 min and kept in a freezer until used.

C. fascians was grown and harvested by the procedures described previously (Hasegawa and King, 1983). Cells were immobilized in acrylamide gel by the procedure of Tosa et al. (1974), blended with a Polytron, and packed in a 2 cm diameter column. The serum was passed through the column at a rate of 100 mL/h at room temperature.

The metabolite was extracted from the treated serum with CH_2Cl_2 by the procedure described previously (Hasegawa et al., 1972a) and isolated by column chromatography on a silica gel. The column was eluted, stepwise, by increasing the concentration of EtOAc in hexane. The major metabolite, thin-layer chromatographically pure, was crystallized from MeOH and analyzed by TLC and NMR spectrum, which was run on a JEOL SP-100 spectrometer. Limonoids were quantitatively analyzed by TLC by the procedure of Maier and Grant (1970).

Nomilin acetyl-lyase activity was also demonstrated with cell-free extracts of *C. fascians*. Two grams of cells was suspended in 50 mL of 0.1 M potassium phosphate buffer (pH 7.0) containing 10^{-3} M dithiothreitol and ruptured with a Branson sonifier, J-22. The suspension was cen-