oil: IR (film) ν 3090, 2980, 2940, 2880, 1720, 1670, 1450, 1370, 1060, 1030, 910, 890 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.22 (s, CH_3), 1.70 (s, vinyl CH_3), 2.54 (t, J = 7.6 Hz, H4), 3.70 (A of AB, J = 8.32 Hz, CH_2O), 3.67 (B of AB, J = 8.32 Hz, CH_2O), 3.9-4.2 (m, CH_2O), 4.7-5.1 (m, vinyl H, 4 H), 5.8-6.0 (m, vinyl H, 1 H); MS 152 (M), 137 (M - Me), 122 (M - 2Me or M - CH_2O), 107 (M - Me - CH_2O), 94 (M - Me - CH_2=$CHCH_3$).

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Registry No. 1, 141-12-8; 2, 70238-37-8; 3, 115306-94-0; 4, 115306-95-1; 5, 115306-96-2; (±)-6, 115362-62-4; (1*R*,6*R*)-6, 96555-02-1; (1*S*,6*S*)-6, 65733-29-1; (±)-7, 115306-97-3; 8, 115306-98-4; (±)-9, 115306-99-5; (±)-10, 115307-00-1; 11, 115307-01-2; 12, 115362-63-5.

Cyclization of 2-(Carbamoyloxy)- and 2-(Sulfamoyloxy)benzoates Mediated by Liver Microsomes[1]

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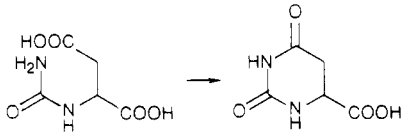
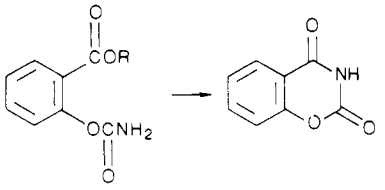
In recent years, the catalytic activity of enzymes[2] has received considerable attention in the synthesis of organic compounds. The use of enzymes for cyclization reactions has been less explored, although these may have enormous interest in view of the mild conditions employed in such reactions. In the biosynthesis[3] of pyrimidine nucleotides, the dihydroorotase-catalyzed ring closure of *N*-carbamoylaspartic acid to dihydroorotic acid is a classical example of enzyme-catalyzed cyclization for the formation of the pyrimidine ring (eq 1). Recently, we reported[4] the



(1)



(2)

1 2

cyclization of 2-(carbamoyloxy)benzoates **1** to 1,3-benzoxazine-2,4-diones **2** by rat liver microsomal fractions (eq 2). The feasibility of this concept prompted us to investigate the enzyme-catalyzed reactions of 2-(sulfamoyloxy)benzoates and 2-(carbamoyloxy)benzophenones as well as the effect on variation of incubation conditions.

Results and Discussion

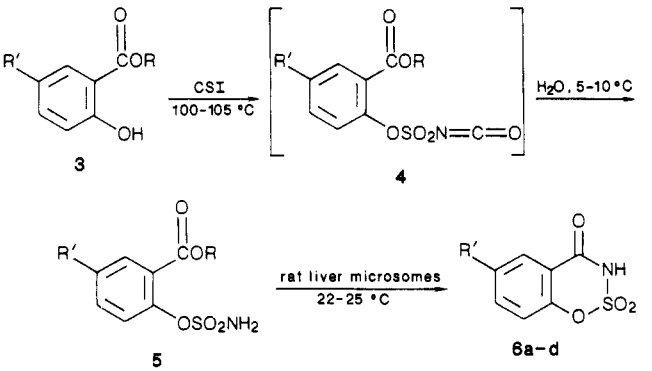
Cyclizations. Our earlier attempts to prepare 4-oxo-3,4-dihydro-1,2,3-benzoxathiazine 2,2-dioxides[5] **6** by thermal cyclization of 2-(sulfamoyloxy)benzoates **5** gave mainly 2-hydroxybenzoates **3** with the cleavage of the OSO_2 linkage. Even the use of bases such as triethylamine and pyridine for cyclization of **5** gave mainly **3** and not the desired **6**. In view of the failure of nonenzymatic cyclizations, it was worthwhile to carry out the enzymatic cyclization of **5**.

Table I. Cyclization of 2-(Sulfamoyloxy)benzoates and 2-(Carbamoyloxy)benzophenones (5 and 8) at 20-25 °C

substrate	R	R′	product	yield[a] of 6a-d and 9a-d, %	mp, °C
5a	CH_3	H	**6a**	74	218-220
5b	C_2H_5	H	**6a**	70	218-219
5c	C_6H_5	H	**6a**	76	218-220
5d	CH_3	Cl	**6b**	81	235
5e	C_2H_5	Cl	**6b**	78	234-236
5f	CH_3	Br	**6c**	71	240-241
5g	CH_3	CH_3	**6d**	75	192-193
5h	C_2H_5	CH_3	**6d**	70	193-195
8a	H	H	**9a**	71	252-255
8b	H	Cl	**9b**	68	274-276
8c	H	CH_3	**9c**	60	248-250
8d	Cl	H	**9d**	75	262
8e	CH_3	Cl	**9e**	66	243-246

[a] Isolated yield of chromatographed product.

Scheme I[a]



[a] R = CH_3, C_2H_5, C_6H_5; R′ = H, Cl, Br, CH_3.

The appropriate precursor, 2-(sulfamoyloxy)benzoate **5**, was prepared by employing chlorosulfonyl isocyanate (CSI). Various alkyl- and aryl-substituted **5** on cyclization with rat liver microsomal fractions at 22-25 °C gave **6** in good yields, Table I (Scheme I). The reactions were monitored by TLC, and it was observed that the enzymatic cyclization of 2-(sulfamoyloxy)benzoates was a slower process than the enzymatic cyclization[4] of 2-(carbamoyloxy)benzoates.

We also investigated the cyclization of 2-(carbamoyloxy)benzophenones **8**. In our earlier studies[6,7] on the reactions of CSI, the reactions of 2-hydroxybenzaldehydes and 2-hydroxyacetophenones with CSI gave 1,3-benzoxazin-2-ones. The reaction of CSI with 2-hydroxybenzophenones produced exclusively 2-(carbamoyloxy)benzophenones **8** and not the cyclized 4-phenylbenzoxazinones **9**. Our efforts to cyclize **8** nonenzymatically, such as by use of bases and thermally, mainly afforded 2-hydroxybenzophenones **7** by the cleavage of the carbamoyloxy function. In view of the above difficulties encountered during attempted cyclizations, we were led to explore the use of biocatalysts. Thus, **8** on cyclization with

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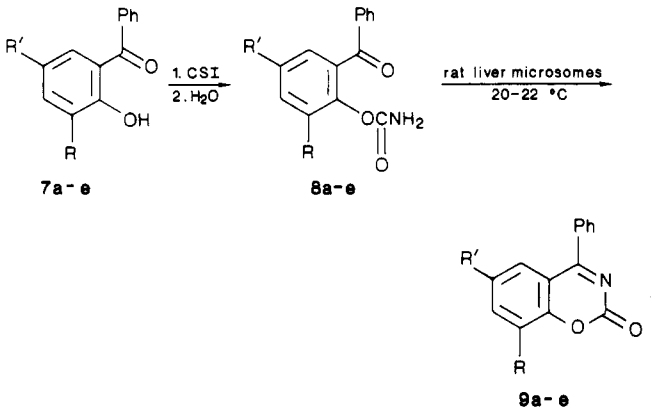
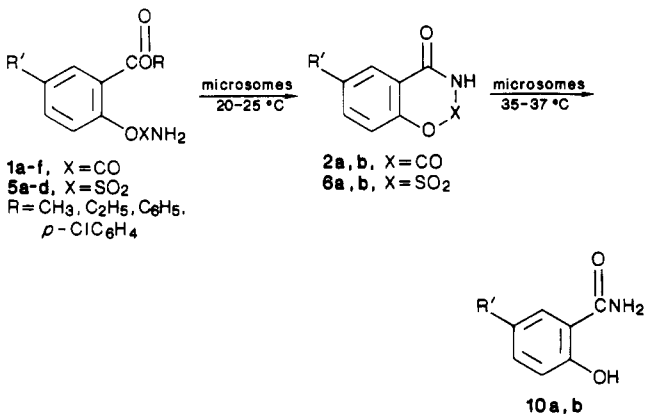
Scheme II[a]



[a] R, R′ = H, Cl, CH_3.

Scheme III[a]



[a] **a**, R′ = H; **b**, R′ = Cl.

rat liver microsomal fractions at 20–22 °C gave 4-phenyl-2*H*-1,3-benzoxazin-2-ones **9** in yields ranging from 60 to 75% (Table I, Scheme II). Starting materials **8** (5–10%) were also recovered in these reactions. The products were characterized by mass spectrometry and analytical and spectroscopic data.

Effect of Temperature. Enzyme-catalyzed amination[8,9] and deamination[10] have been well documented in the literature. During a study of the effect of temperature on the liver microsomal preparation in these reactions, a novel intramolecular amino transfer was observed on carrying out the incubation at 35–37 °C. It is evident from the preceding text that the reaction of 2-(carbamoyloxy)- and 2-(sulfamoyloxy)benzoates performed between 20 and 25 °C produced exclusively cyclized products, i.e., benzoxazinediones **2** and oxodihydrobenzoxathiazine dioxides **6**. However, when these reactions were carried out between 35 and 37 °C, the major product isolated was 2-hydroxybenzamide **10** along with the minor cyclized coproducts **2** and **6** respectively in yields depicted in Table II (Scheme III).

Mechanistically, the formation of **10** is expected to take place via **2** in 2-(carbamoyloxy)benzoates and **6** in the case of 2-(sulfamoyloxy)benzoates. This has been substantiated by investigating a reaction of **1** with rat liver microsomal preparation between 20 and 22 °C for 18 h, the temperature then being raised to 35–37 °C for another 7 h. On workup, this reaction funished **10** as a major product; however, a sample removed after an 18-h period from the reaction carried out between 20 and 25 °C exhibited only the cyclized compound **2**. This has been further confirmed by carrying out a reaction of **2** with microsomes at 35–37 °C, which gives **10**. This fact is clearly observed in Figure 1. The yield of **2b** increased rapidly in the 3-h period to 70%, and the recovery of **1b** was only 10%. The material deficit amounted to 20% at this stage. After the next 2-h period, the yield of **2b** was raised to 80% and the recovery of **1b** decreased to 3%. At 9 h, the yield of **2b** dropped to 64% with no recovery of **1b** while **10b** was observed in 15% yield. At 18 h, the yield of **10b** increased to 75% while **2b** came down to 5% and remained constant thereafter even up to 48 h.

In summary, the cyclizations employing rat liver microsomal preparation provide a new practical approach for the synthesis of 4-oxo-3,4-dihydro-1,2,3-benzoxathiazine 2,2-dioxides **6** and 1,3-benzoxazin-2-ones **9** in good yields.

Table II. Enzyme-Catalyzed Conversions of 2-(Carbamoyloxy)benzoates 1a–f and 2-(Sulfamoyloxy)benzoates 5a–d at 35–37 °C

substrate	R	R′	% yield[a] 2	% yield[a] 10
1a	CH_3	H	8	72
1b	C_2H_5	H	8	75
1c	C_6H_5	H	17	65
1d	p-ClC_6H_4	H	15	63
1e	CH_3	Cl	12	66
1f	C_2H_5	Cl	18	69
substrate	R	R′	% yield[a] 6	% yield[a] 10
5a	CH_3	H	16	53
5b	C_2H_5	H	20	48
5c	CH_3	Cl	17	49
5d	C_2H_5	Cl	24	42

[a] By HPLC employing a Zorbax silica gel column (150 × 4.6 mm), mobile phase, chloroform/butanol (24/1) at 0.7 mL/min flow rate. **2**, **6**, and **10** were calibrated with the standard samples.

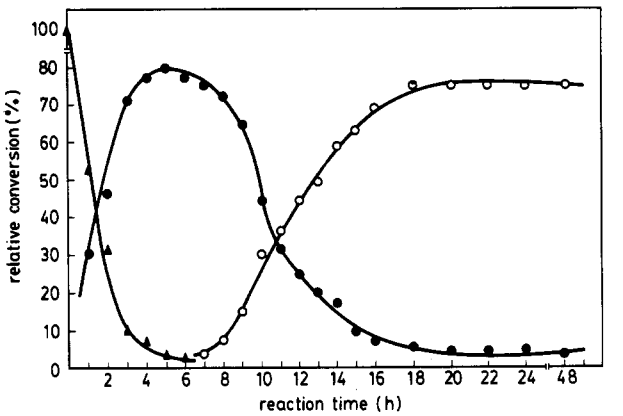


Figure 1. Time course of the incubation mixture of **1b** at 35–37 °C at the indicated times. Percent relative concentration of ethyl 2-(carbamoyloxy)benzoate (▲), 1,3-benzoxazine-2,4-dione **2b** (●), and 2-hydroxybenzamide **10b** (○).

Experimental Section

Melting points were determined on Boetus heating table melting point apparatus and are uncorrected. Proton nuclear magnetic resonance spectra were taken from $CDCl_3$ solution with a JEOL FX90Q FT NMR spectrometer using TMS as an internal reference at 90 MHz. Infrared spectra were recorded on a Perkin-Elmer 283B spectrophotometer. Mass spectra were recorded

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on a VG 7070H mass spectrometer. HPLC analyses were performed with a 6A-Shimadzu instrument with a 254-nm fixed wavelength and Chromatopac C-R3A integrator.

Methyl 2-(Sulfamoyloxy)benzoate (5a). General Procedure. To a stirred solution of methyl 2-hydroxybenzoate (3) (3.5 g, 0.023 mol) in toluene (10 mL) at 100–105 °C was added a solution of chlorosulfonyl isocyanate (2 mL, 0.023 mol) in toluene (3 mL) over a period of 10 min. Stirring was continued for 3 h at this temperature. The toluene was then removed under vacuum, and the residue obtained was added to cold water (30 mL) and left overnight. The solid was filtered, washed with water, and recrystallized from ethanol/methanol to give 4.8 g of **5a** (yield 90%): mp 87–88 °C; IR (KBr) (cm^{-1}) 3400, 3200, 1700, 1370, 1160; ^1H NMR δ 3.89 (3 H, s, OCH_3), 5.6 (2 H, br s, NH_2), 7.25–7.58 (3 H, m, aromatic H), 7.8–7.95 (1 H, dd, aromatic H). Anal. Calcd for $C_8H_9NO_5S$: C, 41.57; H, 3.92. Found: C, 41.43; H, 3.74.

4-Oxo-3,4-dihydro-1,2,3-benzoxathiazine 2,2-Dioxide (6a). General Procedure. A solution of **5a** (50 mg, 0.2 mmol) dissolved in ethanol (20 mL) and 0.02 M phosphate buffer pH 7.4 (15 mL) was added to freshly prepared microsomal suspension (5 mL). Incubation was carried out under aerobic conditions at 22–25 °C for 20 h with gentle shaking. Proteins were precipitated by the addition of acetonitrile (10 mL) to the incubation mixture. The incubation mixture was extracted two times with chloroform (25 mL). The extract was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was dissolved in chloroform/methanol and was subjected to column chromatography (silica gel, chloroform/methanol, 97:3). Further recrystallization from chloroform/hexane furnished **6a** (31 mg, 74%): mp 218–220 °C dec; IR ($CHCl_3$) (cm^{-1}) 3325, 1660, 1400, 1230, 1160; ^1H NMR δ 7.23–7.85 (4 H, m, aromatic H), 13.1–13.5 (1 H, br s, NH). Anal. Calcd for $C_7H_5NO_4S$: C, 42.19; H, 2.53. Found: C, 42.26; H, 2.46.

4-Phenyl-2*H*-1,3-benzoxazin-2-one (9a). General Procedure. To 2-(carbamoyloxy)benzophenone (**8a**) (100 mg, 0.4 mmol) dissolved in ethanol (25 mL) and 0.02 M phosphate buffer pH 7.4 (20 mL) was added freshly prepared microsomal suspension (10 mL). Incubation was performed under aerobic conditions at 20–22 °C for 12 h with mild shaking. Proteins were precipitated by the addition of acetonitrile (20 mL) to the incubation mixture. The incubation mixture was extracted three times with ethyl acetate (30 mL). The organic phase was separated, and the combined organic phases were dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give the crude product **9a**, which was purified by chromatography (silica gel, chloroform/methanol, 98:2), mp 252–255 °C dec (65 mg, 71% yield): IR (KBr) (cm^{-1}) 1720, 1590. Anal. Calcd for $C_{14}H_9NO_2$: C, 75.33; H, 4.06. Found: C, 75.48; H, 3.92.

Reactions of 2-(Carbamoyloxy)- and 2-(Sulfamoyloxy)-benzoates (1 and 5) at 35–37 °C. General Procedure. These reactions were performed in a similar manner as described above at temperatures of 35–37 °C instead of 20–25 °C for 18 h. The residue obtained on workup of the reaction was charged on column chromatography (silica gel, chloroform/methanol, 98:2) to separate 2-hydroxybenzamide **10** from **2** or **6**. 2-Hydroxybenzamide **10** was further purified by recrystallization from chloroform/hexane, mp 138–140 °C, no depression in the mixed melting point and superimposable IR spectra were observed in comparison with the commercial sample of **10**. HPLC analyses showing the ratios of **10/2** and **10/6** are given in Table II.

In all the above reactions with microsomes, a control reaction was monitored under similar reaction conditions employing microsomes preheated at 80 °C for 5 min, which did not afford the products.

Preparation of Liver Microsomal Fraction from Rat. General Procedure. Livers of male Wistar strain rats, 150–220 g, fasted for 1 day before being killed, were homogenized in 0.02 M phosphate buffer (pH 7.4) containing KCl (1.15% w/v). The homogenate was centrifuged at 10000*g* for 30 min, and the resultant supernatant was further centrifuged at 105000*g* for 2 h. The microsomal pellets were resuspended in the same buffer to a final protein concentration of 5 mg/mL determined by the method of Lowry et al.[11]

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Supplementary Material Available: Physical, analytical, and ^1H NMR data for compounds **5**, **6**, and **9** (4 pages). Ordering information is given on any current masthead page.

Ti(O-*i*-Pr)$_4$-Mediated Formation of 2,3-Epithio Alcohols from 2,3-Epoxy Alcohols

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Ti(O-*i*-Pr)$_4$ facilitates the regioselective opening of 2,3-epoxy alcohols and related derivatives with a variety of nucleophiles.[1] As part of ongoing investigations into selective transformations of readily available 2,3-epoxy alcohols,[2] we studied the reaction of 2,3-epoxy alcohols with thiourea mediated by Ti(O-*i*-Pr)$_4$ and have found that good yields of homochiral trans 2,3-epithio alcohols could be obtained regio- and stereoselectively from corresponding epoxy alcohols under mild conditions. Epoxides have been transformed to episulfides by the action of thiourea in aqueous acidic solution followed by basic workup or in methanol solution.[3] Recently, homochiral *trans*-2,3-epoxy-1-hexanol was converted to homochiral *trans*-2,3-epithio-1-hexanol under the former conditions.[4] However, in this reaction a small amount of 1,2-epithio-3-hexanol was also isolated.

As shown in Scheme I, 2,3-epoxy alcohols reacted with thiourea at room temperature or 0 °C in the presence of Ti(O-*i*-Pr)$_4$ in THF. Acidic workup afforded no product from the organic phase. However, when the solution was quenched with saturated aqueous $NaHCO_3$, 2,3-epithio alcohols were obtained. The reaction proceeded with high regio- and stereoselectivity, trans-disubstituted 2,3-epoxy alcohols giving only trans 2,3-epithio alcohols with complete inversion of configuration at both stereogenic centers. The 1,2-epithio alcohols, not detected by TLC and ^1H NMR analyses, were confirmed by synthesizing the 1,2-epithio alcohols from 1,2-epoxy 3-alcohols.[4] The stereochemistry of the reaction was confirmed by correlation with the literature example.[4] For example, from (2*S*,3*S*)-2,3-epoxy-1-hexanol (~94% ee) the corresponding (2*R*,3*R*)-2,3-epithio-1-hexanol (**3a**) (~94% ee) was obtained without loss of enantiomeric excess, which was confirmed by ^1H NMR analysis of the ester derived from (+)-MTPA chloride. In general, trans 2,3-epoxy alcohols gave good yields; however, with cis 2,3-epoxy alcohols the yields were low and thio diols were also formed. Epithiocinnamyl alcohols could also be prepared from the

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