

4-Methanesulfonyloxybutanol: Hydrolysis of Busulfan

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Abstract □ The hydrolysis of busulfan (1,4-dimethanesulfonyloxybutane) was investigated by means of NMR spectroscopy. The final hydrolysis product was found to be tetrahydrofuran. The rather unstable intermediate, 4-methanesulfonyloxybutanol, was synthesized and proved to undergo cyclization to tetrahydrofuran by intramolecular alkylation. The half-life of this first-order reaction in aqueous solution at 37° was determined to be approximately 12 min. at pH 3 as well as at pH 7.4. From the present data, it is concluded that 4-methanesulfonyloxybutanol is unlikely to be responsible for the biological action of busulfan.

Keywords □ Busulfan—hydrolysis, NMR determination of hydrolysis intermediate as 4-methanesulfonyloxybutanol □ 4-Methanesulfonyloxybutanol—synthesis, identified as an unstable intermediate in the hydrolysis of busulfan, NMR □ NMR spectroscopy—identification of 4-methanesulfonyloxybutanol as an unstable intermediate in the hydrolysis of busulfan

In connection with an investigation of the genetic action of busulfan (1,4-dimethanesulfonyloxybutane), it has been found that aqueous solutions of hydrolyzed busulfan, as well as solutions of a compound assumed to be 4-methanesulfonyloxybutanol (III), induced mutations to a greater extent than busulfan itself (1). Compound III, formerly a monofunctional alkylating agent, was of potential interest as an intermediate in the hydrolysis of busulfan with reference to the discussion of the mechanism of the biological effects of busulfan. To our knowledge, the synthesis and stability of III have not been described in the literature¹. For this reason, it seemed justified to synthesize the intermediate III and to investigate its behavior. Furthermore, the partial saponification and hydrolysis of busulfan were followed by NMR spectroscopy.

EXPERIMENTAL²

Synthesis—4-Benzoyl-1-methanesulfonyloxybutane (II)—To a stirred mixture of 4-benzyloxybutanol (I) (2) [9 g., 0.05 mole, b.p. 157–160° (12 mm.)] and methanesulfonyl chloride (6.9 g., 0.06 mole), pyridine (20 ml.) was added dropwise while keeping the temperature below 40° by cooling. After additional stirring for 1 hr., the reaction mixture was poured into a mixture of ice and 4 N HCl. It was extracted with chloroform and the organic layer was washed with 4 N HCl, dried (magnesium sulfate), and evaporated *in vacuo*. The residue was suspended in water, and the excess methanesulfonyl chloride was hydrolyzed at room temperature at pH 7 using an automatic end-point titrator (11.2 ml. of 1 N NaOH).

¹ The authors were not successful in obtaining the details of the original method of preparation of the compound (I) alleged to be the monomethyl derivative III.

² The NMR spectra were obtained on a Varian A-60 A spectrometer. The titration curves were obtained on a Radiometer titrator, Type TTT 1a, equipped with a Radiometer titrator, SBR 2b.

It was extracted with chloroform, washed with water, dried (magnesium sulfate), and evaporated *in vacuo*, yielding crude II (12 g., 93%). Crude II (3 g.) was chromatographed on a dry silica gel column³. The fractions, shown by TLC³ to be homogeneous, were combined and evaporated *in vacuo*. After drying of the residue *in vacuo* (1 mm.) for 2 hr. at 22°, II was obtained as a colorless oil (2.1 g.); NMR (CDCl₃, tetramethylsilane): CH₂CH₂ (m, 1.78, 4H), CH₂OSO₂ (s, 2.94, 3H), CH₂CH₂O (t, 3.52, 2H), CH₂OSO₂ (t, 4.26, 2H), C₆H₅CH₂O (s, 4.51, 2H), and C₆H₅ (s, 7.36, 5H).

Anal.—Calc. for C₁₇H₁₈O₄S (mol. wt. 258.32): C, 55.80; H, 7.02; S, 12.38. Found: C, 55.67; H, 7.04; S, 11.92.

4-Methanesulfonyloxybutanol (III)—A solution of II (1 g., 3.9 mmole) in dry ethanol (15 ml.) was hydrogenated at room temperature after addition of 10% palladium-on-charcoal (0.15 g.). After the uptake became negligible, the catalyst was removed by filtration. The filtrate was evaporated *in vacuo* in a bath not exceeding 15° to yield III as a colorless oil. All operations including analyses were performed within 6 hr., and III was kept below –10° when possible. The NMR spectrum of III is given in Fig. 1.

Anal.—Calc. for C₇H₁₂O₄S (mol. wt. 168.21): C, 35.70; H, 7.20; S, 19.05. Found: C, 35.46; H, 7.59; S, 18.92.

Half-Life Studies—The half-life of III was obtained by titration of liberated methanesulfonic acid in 0.1-mmole solution of III at pH 7.4 and 3, respectively, using 0.33 N NaOH. The titration curve was recorded until the sodium hydroxide consumed had become negligible. The reaction order and the half-life were determined as usual.

Hydrolysis of Busulfan—Acidic Conditions—A suspension of busulfan (0.025 g., 0.1 mmole) in deuterium oxide (D₂O) (0.4 ml.) was kept in a sealed NMR test tube at 70° and shaken occasionally until a solution was obtained (24 hr.). The NMR spectrum was recorded to show the signals of IV. The formation of IV was verified and the absence of 1,4-butanediol was proved by NMR spectra taken after the addition of IV and the diol successively to the de-sealed NMR tube.

Alkaline Conditions—The experiment was performed as described under *Acidic Conditions*, except that 2.4 N NaOD (0.1 ml.) was added to the suspension prior heating to show IV as the reaction product.

Partial Saponification—To a mixture of busulfan (0.125 g., 0.5 mmole), D₂O (1 ml.), and (CD₃)₂CO (3 ml.), 2.4 N NaOD (0.2 ml., 0.48 mmole) was added and the mixture was refluxed for 4 hr. The NMR spectrum of the still alkaline reaction mixture was examined, and the presence of IV along with the starting material was proved as described under *Acidic Conditions*.

RESULTS AND DISCUSSION

Experiments to provide 4-methanesulfonyloxybutanol (III) by partial saponification of busulfan, as well as total hydrolysis under acidic and alkaline conditions, were followed by NMR spectroscopy. No reaction products but tetrahydrofuran (IV) could be detected. Fast cyclization of III to IV by intramolecular alkylation was the only explanation for this result and was consistent with the described cyclization of partially mesylated or tosylated polyalcohols under acidic (3, 4) and alkaline (5) conditions. The same type of cyclization occurs during the recently reported (6) formation of

³ HF₂₅₄, Merck; C₆H₁₈ (three parts), ethyl acetate (one part); detection by UV.

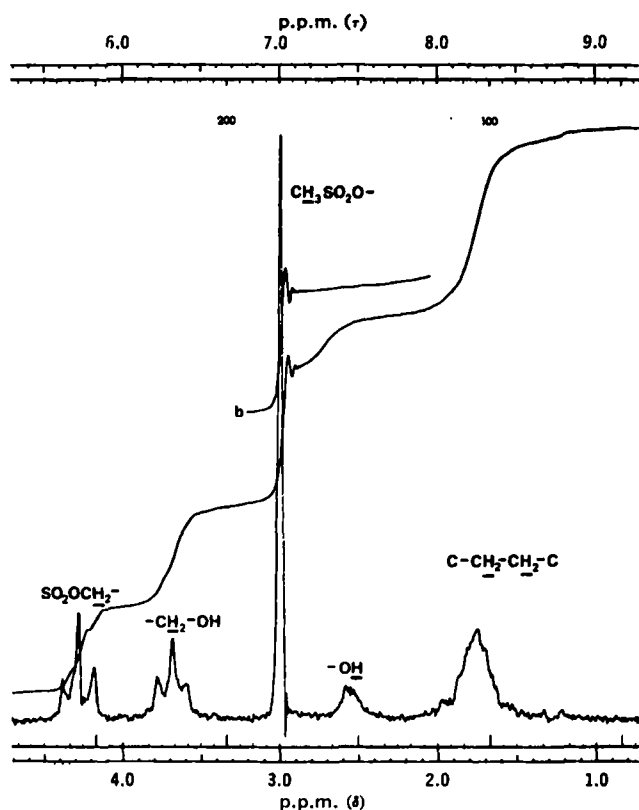
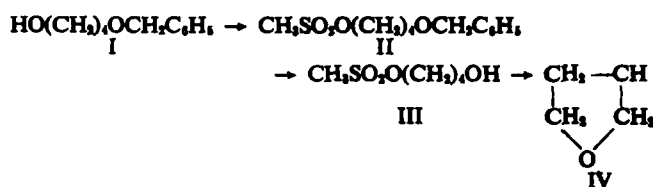


Figure 1—NMR spectrum of III, 10% (CDCl_3 , tetramethylsilane). The integral b is recorded after shaking the NMR solution with D_2O .

2,5-dimethyltetrahydrofurans from their corresponding 2,5-bis-methanesulfonyloxyhexanes. For these reasons, the synthesis of III was based on a temporary protection of the hydroxyl function as outlined in Scheme I. The reductive debenzoylation as the last step resulting in III was advantageous since it could be performed under mild conditions to yield III quantitatively as a colorless, analytically pure, and unstable oil. The structure was proved by its NMR spectrum in CDCl_3 (Fig. 1). When the NMR test tube was left for 46 hr. at room temperature, an almost quantitative for-



Scheme I

mation of IV and methanesulfonic acid was observed. In water, III has a half-life of approximately 12 min. at 37° both at pH 7.4 and 3, and it was transformed to IV both in the neutral and acidic solutions in a first-order reaction. The cyclization could conveniently be followed by titration of liberated methanesulfonic acid.

The presented results proved that the hydrolysis of busulfan leads to tetrahydrofuran (IV) through the intermediate III, which is in contrast to the reported formation of 1,4-butanediol (7). When one considers the high rate of cyclization of the monomethyl compound, III, it is unlikely that this compound is responsible for the biological action of busulfan.

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