The Synthesis of Some Tritium-labelled Mutagenic Alkyl Alkanesulfonates

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Certain alkyl alkanesulfonates, especially ethyl methanesulfonate, have been shown to induce a high frequency of gene mutation. In higher plants, the mutagenic efficiency of ethyl methanesulfonate, *i.e.* the highest mutation rate which can be obtained, is around five times higher than the corresponding efficiency of ionizing radiations.[1] This is due to the low toxicity of this substance and to the fact that its chromosome breaking ability is relatively limited. Methyl methanesulfonate is appreciably more toxic and induces a high frequency [2] of chromosomal aberrations, and has therefore a limited mutagenic efficiency. The various propyl and butyl methanesulfonates exhibit further interesting variations in the patterns of biological effects, from high mutagenic efficiency of the propyl, butyl and isopropyl esters to complete lack of mutagenic power at a practically unchanged toxicity in the isobutyl ester.[3]

Although the reactions of methyl and ethyl methanesulfonates with DNA have been studied in some detail,[4] the causes of the differences in their biological activities are not well understood.

The great dissimilarities between homologous and isomeric alkyl alkanesulfonates with respect to their biological effects have to be ascribed to a number of factors influencing uptake and distribution in the tissue, reaction rates with different nucleophilic molecules,[5,3] and the later fate of the alkylated groups.[4]

For quantitative biological and biochemical studies of the action mechanisms of the alkyl alkanesulfonates it was found necessary to label the alkyl group. Since a high specific activity is necessary, as well as a high resolving power in autoradiographs, T-labelling was preferred to the ^{14}C-labelling used in the studies of Brookes and Lawley.[4]

In the present work, methyl-T methanesulfonate, ethyl-2-T methanesulfonate and isopropyl-1,3-T methanesulfonate were synthesized on a semi-micro scale in high yields from commercially available tritiated alcohols, which were allowed to react with methanesulfonic anhydride. The synthesis and purification of the esters was performed on a vacuum line, as described in the experimental part. Suitable reaction conditions were worked out using inactive alcohols on a somewhat larger scale. The yield of the highly reactive isopropyl ester was considerably improved when the reaction was performed in the presence of the sterically hindered base, 2,6-dimethylpyridine. The tritiated esters were stored as 0.1 % solutions in dry light petroleum to minimize autodecomposition, which was determined, and to facilitate withdrawal of small samples from the stock solution. The specific activities of the esters prepared are identical with those of the parent alcohols which, in principle, may be obtained at any desired value.

Experimental. Methanol and ethanol of analytical grade were used. Isopropanol (C.p.) and light petroleum (b.p. 40–60°) were dried over calcium hydride and distilled. 2,6-Dimethylpyridine was dried over potassium hydroxide and distilled.

The tritiated alcohols were obtained from The Radiochemical Centre, Amersham, Bucks., England. All work involving transference of radioactive material was carried out on a vacuum line at a pressure of 10^{-4} mm Hg, using an oil pump backing a mercury vapour diffusion pump fitted with a mercury trap. The vacuum line was constructed essentially as described by Calvin *et al.*[6]

Methanesulfonic anhydride was prepared according to the method of Owen and Whitelaw.[7] It was redistilled immediately before use (m.p. 70–71°, b.p. 137–139°/10 mm).

Ethyl methanesulfonate. Methanesulfonic anhydride (3.5 g, 0.02 mole) and dry ethanol (0.92 g, 0.02 mole) were refluxed with exclusion of moisture for 1 h at a bath temperature of 150°. The reaction mixture was distilled and the fraction b.p. 87–89°/10 mm was collected. Yield 1.86 g (0.015 mole, 75 %); n_D^{25} 1.4164 (lit.[8] b.p. 90°/10 mm; n_D^{15} 1.4194). The ester obtained here as well as a sample prepared on a microscale using the vacuum line procedure were shown to give single peaks when analysed by gas chromatography, as described below.

Ethyl-2-T methanesulfonate. Ethanol-2-T (6.45 mg, 0.140 mmole at 713 mCi/mmole) was condensed on the vacuum line into a 15 ml tube fitted with a tap and containing methanesulfonic anhydride (45 mg, 0.26 mmole), the

tube being cooled in liquid nitrogen. The tube was sealed and heated at 90° for 2 h. The reaction tube was then connected to the vacuum line *via* two U-tubes coupled in series. The ester was distilled slowly at 20°C and 10^{-4} mm into the first U-tube, which was cooled in liquid nitrogen. Traces of crystalline anhydride were removed from the distillate by means of another distillation into the second U-tube. This tube, when cooled as above, was sealed off, and filled to atmospheric pressure with dry argon from the vacuum line. It was then removed, cleaned at the glass joint and closed with a glass stopper before weighing. Yield of ethyl-2-T methanesulfonate, 17.7 mg (0.142 mmole). The ester was stored as a 0.1 % solution in dry light petroleum (b.p. 40–60°).

Methyl methanesulfonate. Methanesulfonic anhydride (2.97 g, 0.017 mole) and dry methanol (0.69 ml, 0.017 mole) were refluxed in a 25 ml flask for 1 h at 150°. The reaction mixture was distilled and the fraction b.p. 102–103°/25 mm was collected. Yield 1.23 g (0.0112 mole, 66 %); n_D^{25} 1.4127 (lit.[8] b.p. 96–98°/19 mm; n_D^{20} 1.4150). The purity of the ester was confirmed by gas chromatography.

Methyl-T methanesulfonate. The synthesis was performed as described for the ethyl-2-T ester. Methanol-T (5.33 mg, 0.166 mmole at 150 mCi/mmole) was reacted at 90° for 2 h with methanesulfonic anhydride (45 mg, 0.26 mmole). The yield of methyl-T methanesulfonate was 18.5 mg (0.168 mmole). The methyl ester was stored as described above for its ethyl homologue.

Isopropyl methanesulfonate. Methanesulfonic anhydride (13.1 g, 0.075 mole), isopropanol (3.05 g, 0.05 mole) and 2,6-dimethylpyridine (2.4 g, 0.025 mole) were heated at 50° for 3 h in a 50 ml flask fitted with a reflux condenser and protected from moisture. The ester was distilled and the fraction boiling at 39–41°/0.01 mm (57–58°/0.7 mm) was collected.

Yield 5.9 g, 0.0425 mole (85 % on isopropanol); n_D^{25} 1.4173 (lit.[9] n_D^{25} 1.4167). The purity of the ester was checked by gas chromatography. The ester was kept in an atmosphere of dry argon and stored at −30°C. The synthesis was worked out on a semimicro scale (10–60 mg isopropanol), using a vacuum line procedure as described above. Optimum yields of ester (85–92 %) were obtained using molar proportions 1.5:1:0.5 of anhydride, isopropanol, and dimethylpyridine, respectively, at a temperature of 50° and a reaction time of 2.5–3 h.

Isopropyl-1,3-T methanesulfonate. 2,6-Dimethylpyridine (8.1 mg, 0.083 mmole) was condensed on the vacuum line into a 15 ml tube fitted with a tap and containing methanesulfonic anhydride (43 mg, 0.25 mmole), the tube being cooled in liquid nitrogen. Isopropanol-1,3-T (9.1 mg, 0.151 mmole at 66 mCi/mmole) was similarly condensed into the reaction tube. The tap was closed and the tube removed and heated in an oven at 50°C for 2.5 h. The product formed was isolated and purified as described above for the ethyl ester. The yield of isopropyl-1,3-T methanesulfonate was 19.2 mg, (0.139 mmole). The ester was stored at −30°C as a 0.1 % solution in dry light petroleum (b.p. 40–60°).

Special analyses. Gas chromatography. The analyses were performed on a Perkin-Elmer Fraktometer 116 with a hot wire detector using a polyethylene glycol succinate on carbowax column. At a column temperature of 140°C and a helium flow rate of 32 ml/min, the following retention times were noted:

Methyl methanesulfonate	58.5 min
Ethyl methanesulfonate	61.5 min
Isopropyl methanesulfonate	50.25 min

Radiation chemical stability. In order to evaluate the radiation chemical stability of alkyl-T alkanesulfonates under different conditions, solutions of ethyl-T methanesulfonate in dry and wet light petroleum or dichloromethane were irradiated with doses of external ^{60}Co γ-radiation corresponding to 0.12 and 1 year self-irradiation. At 0.7 Ci/mmole and a concentration of 2 mg/g solvent, the yearly self-irradition dose is around 1.2×10^6 rad. When this γ-radiation dose was given for 4 h, the decomposition, as measured by the formation of free acid was:

0.25 % in dry light petroleum
1 % in wet light petroleum
2 % in dry CH_2Cl_2
4 % in wet CH_2Cl_2.

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A Convenient Method for the Quantitative Determination of Sulfoxides

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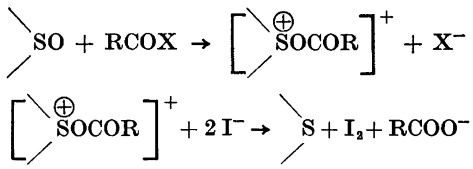
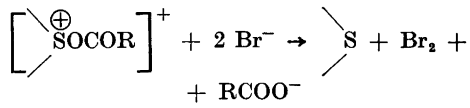
The methods available for quantitative determination of sulfoxides are mainly based on reduction of the sulfoxide group. Thus, the reduction by means of hydrogen bromide in glacial acetic acid [1] has often been used and studied,[2,3] and other reducing agents such as titanium trichloride [4,5] and stannous chloride [6] have been found valuable. Other methods are also known, such as the potentiometric titration of sulfoxides as bases with perchloric acid in acetic anhydride.[7]

During work on the reduction of sulfoxides, the present author found an accurate and rapid method for the quantitative determination of such compounds. The method, which consists in acylation of the sulfoxide in the presence of iodide, involves the intermediate formation of an acyloxysulfonium salt and can be represented by the following scheme:



With bromide, free bromine is formed:



As acylating agent acetyl chloride was used. For analytical purposes acetic acid was found to be a good reaction medium because of the formation of a one-phase system on adding dilute hydrochloric acid. The iodine was titrated with sodium thiosulfate. Details are described in the experimental part.

The method seems to be generally applicable for compounds in which the sulfoxide group is not too sterically hindered and the reaction at room temperature is usually complete within some minutes. In Table 1 the equivalent weights found for some various compounds are represented.

Acyloxysulfonium salts have earlier been proposed by some authors [8-12] as intermediates in the reaction between acid anhydrides and sulfoxides,[13,14] and recently in a reaction consisting in oxidation of alcohols in dimethyl sulfoxide - acid anhydride mixtures.[15]

Experimental. Procedure. The following method was found to be suitable: 0.5—1.0 mmole of the sulfoxide compound is accurately weighed, transferred to a 100 ml Erlenmeyer flask, and dissolved in 20 ml of glacial acetic

Table 1. Equivalent weights found for some different sulfoxides.

Sulfoxide	Reaction time min	Equivalent weights found	calc.
$(CH_3)_2SO$	2	39.4	39.1
$(C_6H_5CH_2)_2SO$	2	114.6	115.2
$CH_3CH_2CH_2SOCH_2CH_2CO_2H$	2	82.4	82.1
$C_6H_5CH_2SOCH_2CH_2CO_2H$	2	106.2	106.1
$CH_3SOC(CH_3)_2CO_2H$	5	75.1	75.1
$CH_3SOC(CH_3)_2CH_2CO_2H$	5	81.8	82.1