PREPARATION OF [1-^{14}C] and ^{35}S-LABELLED ALKYL SULPHONATES

Andrew J. Taylor and Anthony H. Olavesen
Department of Biochemistry, University College,
P.O. Box 78, Cardiff, CF1 1XL, U.K.
and Colin James
Unilever Research, Colworth House, Sharnbrook,
Bedford, MK44 1LQ, U.K.
Received November 29, 1976
Revised February 18, 1977

SUMMARY

Undecyl, dodecyl and hexadecyl sulphonates were prepared with ^{14}C-label in the 1-position. The syntheses require the formation of a Grignard intermediate from the appropriate n-1 alkyl bromide followed by carboxylation of the Grignard with $^{14}CO_2$ and subsequent esterification with diazomethane. Reduction of the ester with lithium aluminium hydride and conversion to the corresponding alkyl bromide provides a suitable 1-^{14}C labelled derivative for conversion to the required thiol which is readily oxidised to the corresponding sulphonate with retention of the label in the 1-position. The synthetic route provides an acceptable overall yield (18-24%) of specifically labelled alkyl sulphonates of high purity. By using [^{35}S]thiourea in the sequence ^{35}S-labelled dodecyl sulphonate was readily obtained. The identity and purity of the products was confirmed by mass spectrometry, infra-red spectroscopy, t.l.c. and reverse-isotope dilution.

Key words: 1-carbon-14, sulphur-35, undecyl, dodecyl, hexadecyl, sulphonates

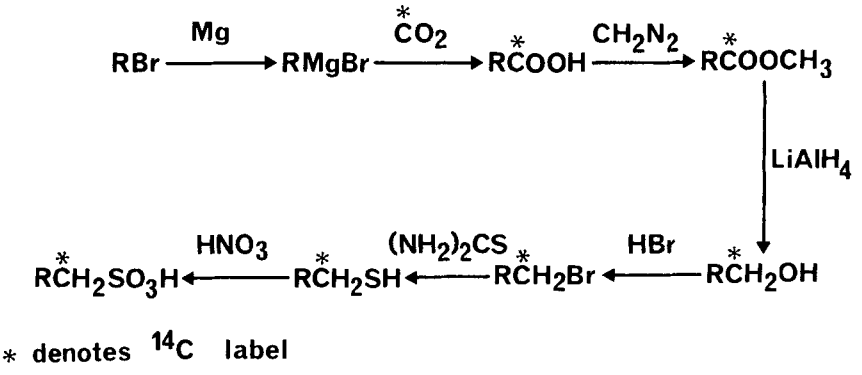
INTRODUCTION

Alkyl sulphonates are constituents of some commercial detergent formulations and in order to investigate their metabolic fate in animals, three homologues (undecyl, dodecyl and hexadecyl sulphonates) were synthesized with a ^{14}C-label in the 1-position of the alkyl chain. Additionally, dodecyl sulphonate was labelled with ^{35}S to study possible desulphonation of this compound *in vivo*. Sulphonation reactionsare traditionally those of the detergent industry for the production of alkyl and arylalkyl sulphonates.

0362-4803/78/0214-0249$01.00/0

The methods employed by industry are unsuitable for laboratory preparations because they produce a range of homologues and isomers that are not readily separable. Of the methods available for the production of primary alkyl sulphonates (1), the oxidation of alkyl thiols is the most suitable as no secondary or poly-sulphonate by-products are formed. The respective alkyl thiols were synthesized by a route (see Figure) which produced an acceptable overall yield (18-24%) and specifically-labelled products of high purity. The combination of previously reported procedures for the preparation of long chain [1-^{14}C]alkyl compounds (2,3) with the sulphonation procedure described above, yielded [1-^{14}C]alkyl sulphonates of sufficient purity (>98%) and specific activity (1.9-5.1 mCi/mmole) for metabolic studies. Dodecyl [^{35}S] sulphonate was prepared by reacting ^{35}S-thiourea with dodecyl bromide and subsequent oxidation of the resultant thiol with 70% HNO_3. Thus, by an extension to and slight modifications of established methods (2,3), a general procedure is made available for the preparation of specifically labelled alkyl sulphonates.

Figure Scheme for preparation of [1-^{14}C]alkyl sulphonates



MATERIALS

Decyl bromide and pentadecyl bromide were obtained from Eastman Kodak Ltd., Kirkby, Liverpool, U.K. Undecyl bromide was a product of Pflatz & Bauer Inc., Flushing, New York, U.S.A. and B.D.H. Ltd., Poole, Dorset, U.K. supplied dodecyl bromide and the other common reagents. The alkyl bromides were redistilled in vacuo before use. $Ba^{14}CO_3$(20-25mCi/mmol) and ^{35}S-thiourea (6.3mCi/mmol) were supplied by The Radiochemical Centre, Amersham, Bucks, U.K.

METHODS

Preparation of [1-^{14}C]Alkyl Bromides

The [1-^{14}C]labelled alkyl bromides were prepared by slight modifications to methods previously reported (2,3). A Grignard reagent was prepared from the appropriate alkyl bromide (0.01mol) and subsequently carbonated with 5mCi $Ba^{14}CO_3$ together with unlabelled $BaCO_3$ (total $BaCO_3$ 0.001 mol) to yield the [1-^{14}C]alkanoic acids in a radiochemical yield of 80-85%, based on $Ba^{14}CO_3$.

Methylation of the [1-^{14}C]alkanoic acids with diazomethane (4) yielded the respective methyl esters which on analysis by radio g.l.c. (3% SE-30 column, 5' x 4mm, 150-185^{o}C) were shown to be >90% radiochemically pure. Reduction of the methyl esters with $LiAlH_4$ produced the corresponding [1-^{14}C] alkyl-1-ols in 75% radiochemical yield. T.l.c. of the products on Kieselgel-H plates in n-hexane:ether:acetic acid (40:10:1, by vol.) followed by radioautography showed that >90% of the applied radioactivity cochromatographed with samples of authentic alcohols. Conversion to the respective [1-^{14}C]labelled alkyl bromides was carried out in 58-62% radiochemical yield by treatment of the [1-^{14}C] alcohols with excess hydrobromic acid. The products were shown to be homogeneous by t.l.c. as described above.

Preparation of [1-^{14}C]Thiols

The alkyl bromides were converted to the corresponding thiols by refluxing with thiourea in absolute ethanol for 6h. (Typical proportions were 40mg thiourea:270µl absolute ethanol for 0.5mM). After cooling, NaOH (approx. 30mg in 30µl water) was added and the mixture was refluxed for a further 3h. Acidification of the mixture with a few drops of 1M-H_2SO_4 was followed by immediate extraction of the thiol into ether to prevent acid hydrolysis. The ether extracts were washed with water and dried over anhydrous Na_2SO_4, ether was removed under N_2 and the resultant thiol was weighed. Unlabelled thiol was added so that the specific activity was at least 10µCi/mg (e.g. 1.9mCi/mmole undecanethiol).

Preparation of Sodium [1-^{14}C]Alkyl Sulphonates

An excess of 70%HNO_3 (0.8-1.2ml is sufficient for the oxidation of up to 100mg alkyl thiol) was stirred and heated while the alkanethiol was added slowly through a side-arm. The mixture was heated at 45^{o}C for 3h then degassed at 90^{o}C by bubbling N_2 until no more brown fumes were observed. Neutralization with 30% w/v aqueous NaOH and storage overnight at 4^{o}C, produced white crystals which were filtered and recrystallized twice from boiling ethanol. Approximately 50mg of each alkyl sulphonate was obtained, which represents an overall radiochemical yield of 18-24%. The specific radioactivities of the labelled alkyl sulphonates were undecyl sulphonate 1.9mCi/mmole, dodecyl sulphonate 2.7mCi/mmole and hexadecyl sulphonate 5.1mCi/mmole.

Preparation of Sodium dodecyl [^{35}S] Sulphonate

Sodium dodecyl [^{35}S] sulphonate was prepared by refluxing equimolar proportions of dodecyl bromide and ^{35}S-thiourea (125mg and 40mg respectively) in absolute ethanol (270µl) for 6h, cooling and neutralizing with NaOH (28mg in 28µl water). The mixture was refluxed for a further 3h and then

extracted and oxidized in the manner described for the ^{14}C-labelled compounds. Approx. 30mg of dodecyl sulphonate was synthesized with a specific activity of 1.6mCi/mmole on the day of synthesis.

ANALYSIS OF PRODUCTS

Thin layer chromatography

The homogeneity of the radioactive alkyl sulphonates was demonstrated by t.l.c. in the following solvent systems:- silicagel-H; butan-1-ol: acetic acid:water, 4:1:1, by vol.; chloroform:methanol:water, 65:25:4, by vol.; and a reverse phase system, silicagel plates were impregnated with 10% "Nujol" in hexane, dried, spotted and developed in methanol:water saturated with "Nujol", 7:3, by vol. The three alkyl sulphonates were shown to be homogeneous in all three solvent systems.

Reverse Isotope Dilution Analysis

Recrystallization of labelled alkyl sulphonates with excess unlabelled alkyl sulphonates from ethanol and subsequent radioactive measurements, showed that the synthesized compounds were >98% radiochemically and chemically pure.

Infrared Spectrometry

The alkyl sulphonates were examined as KBr discs in a Perkin-Elmer 257 Grating Infrared spectrometer. Typical absorption bands at 2850cm^{-1} and 1470cm^{-1} ($-CH_2-$), 1470cm^{-1} and 1380cm^{-1} ($-CH_3$), 1150-1260cm^{-1} and 1010-1080cm^{-1} (sulphonate group) were observed for all three alkyl sulphonates.

Mass Spectrometry

Spectra of the alkyl sulphonates were obtained by field desorption mass spectrometry by Dr. D. E. Games, Chemistry Dept., University College, Cardiff on a Varian CH5 double focussing machine. Cluster ions of the form M+Na, 2M+Na etc. were observed with this technique (4). Undecyl sulphonate produced a major ion at m/e 281 (M+Na), dodecyl sulphonate gave ions at m/e 295 (M+Na) and m/e 567 (2M+Na) while the spectrum of hexadecyl sulphonate

contained a M+Na ion at m/e 351.

AJT is grateful to the Science Research Council and Unilever Ltd. for a C.A.S.E. studentship.

REFERENCES

1. Gilbert, E.E. - Sulphonation and Related Reactions, John Wiley, New York (1965)
2. Dauben, W.G. - J. Amer. Chem. Soc. 70: 1376 (1948)
3 Jerchel, D., Becker, H. & Schmeiser, K. - Z. Naturforschung 9b: 169 (1954)
4. De Boer, Th. J. - Rec. Trav. Chim. 73: 229 (1954)
5. Games, D.E. - Biochem. Soc. Trans. 3: 455 (1975)