

NOTES

SYNTHESIS OF n-HEXADECANE-1-T

Tritiated water and tritium and carbon-14 labelled toluene or n-hexadecane are used extensively for efficiency determinations in liquid scintillation counting, either by internal standard, sample channel ratio or external standard ratio methods. In this laboratory, labelled n-hexadecane is preferred to labelled toluene, mainly because it is much less volatile than the latter compound, and also because it can be easily combusted to check the performance (recovery, spill-over and memory) of the Packard Sample Oxidizer, model 306 (Packard Instrument Co., Inc., Downers Grove, Illinois, USA).

Calibrated n-hexadecane-1,2(n)-T (and n-hexadecane-1-C¹⁴) can only be obtained from The Radiochemical Centre, Amersham, England; however, the specific activity of this tritiated compound is rather low (approximately 1.4 $\mu\text{Ci/ml}$). Since only 20-30 μl samples of labelled n-hexadecane can be used with the Sample Oxidizer, long counting times are needed to obtain optimal counting statistics (standard deviation 0.1 % or less), especially since counting efficiencies of tritiated samples range from 15 to 25 %, as measured in a Packard 3390 Tri-Carb liquid scintillation spectrometer with 544 Absolute Activity Analyzer. Also, we frequently prepare sets of standardized quenched samples (each containing 100 μl of labelled n-hexadecane) to determine counting efficiencies in single- or double-labelled experiments, which is a rather expensive procedure. Therefore, both carbon-14 and tritium labelled n-hexadecane of higher specific activity were needed. n-Hexadecane-1-C¹⁴ was obtained from The Radiochemical Centre and diluted with carrier n-hexadecane (Fluka AG, Buchs, Switzerland) to a specific activity of 5.0 $\mu\text{Ci/ml}$. Since tritiated n-hexadecane of high specific activity was not available, we looked for a simple and convenient method for the synthesis of this compound.

n-Hexadecane-1,2(n)-T is prepared by catalytic reduction of n-hexadec-1-ene with tritium gas (1). Gordon and Van Klaveren (2) obtained tritiated n-hexadecane by exchange between tritiated water and n-hexadecane with a Co-Mo-S catalyst; however, both methods are not easily applied in the average tracer laboratory. Also, the methods of Carey and Smith (3) (reduction of cetyl iodide with either Zn and acetic acid, Zn-Cu and ethanol or by hydrogenation on Pd-CaCO₃) and of Levene (4) (treatment of cetyl iodide with Zn in acetic acid, saturated with hydrogen chloride) are difficult to adapt for tritiation of the compound. The

reaction schemes of Schmidt and Bubner (5) and of Klimashevskaya *et al.* (6), as used for the preparation of n-hexadecane-1-C¹⁴ (reduction of palmitic acid to cetyl alcohol, which is transformed into cetyl bromide or cetyl iodide, followed by reduction of these compounds with LiAlH₄ or Zn-acetic acid-hydrogen chloride, respectively) could also be used for the synthesis of tritiated n-hexadecane, starting from palmitic acid-9,10(n)-T. These methods, however, are rather lengthy.

Finally, two methods for the synthesis of tritiated n-hexadecane were investigated; decomposition of cetyl magnesium bromide with tritiated water, or reduction with tritiated LiAlH₄ of a suitable C₁₆-compound, e.g. cetyl bromide (5), cetyl iodide (7), sodium n-hexadecyl sulfate (8) and n-hexadecyl p-toluenesulfonate. n-Hexadecanol (cetyl alcohol), cetyl bromide and LiAlH₄ were obtained from Fluka AG. Cetyl iodide (E. Merck, Darmstadt, West-Germany) was redistilled in vacuum. Sodium n-hexadecyl sulfate was prepared with the method of Evans (9); for the synthesis of n-hexadecyl p-toluenesulfonate, the method of Marvel and Sekera (10) was used. Anhydrous diethyl ether and tetrahydrofuran were obtained by refluxing with sodium or LiAlH₄, respectively. All reactions were carried out under nitrogen. Reaction mixtures were analyzed on a Pye 104 gas chromatograph, with a 150 cm column of 3 % OV-1 on Gas-Chrom Q (100-120 mesh) (Applied Science Laboratories, State College, Pennsylvania, USA) at 170°C. The carrier gas was nitrogen at a flow rate of 60 ml/min; retention times were 2.0 min for n-hexadecane, 6.0 min for n-hexadecanol, 8.6 min for cetyl bromide and 13.2 min for cetyl iodide.

Since tritiated water of high specific activity is available at low cost, decomposition of cetyl magnesium bromide with HTO seemed the most appropriate method for the synthesis of n-hexadecane-1-T. GLC analysis of preliminary experiments with 50 mmol of Grignard reagent, prepared in 100 ml or 500 ml of diethyl ether and decomposed with water, indicated that 50-70 % of n-hexadecane was formed. However, a by-product, which crystallized from the ether extract, was identified as n-dotriacontane (C₃₂H₆₆) by m.p., mixed m.p., GLC at 245°C and mass spectrometry (M⁺ = 450). Therefore, this method was not further investigated.

We next examined the reduction with LiAlH₄ of cetyl bromide, cetyl iodide and the sodium sulfate and tosyl derivatives of cetyl alcohol. Theoretically, a molar ratio of 1:0.25 of substrate to LiAlH₄ should be sufficient for complete conversion of these compounds into n-hexadecane. The results in Table I indicate that, by using a 10 % excess of LiAlH₄ (molar ratio 1:0.28), only half of the cetyl iodide and cetyl bromide were reduced to n-hexadecane. Therefore, reaction conditions were sought in which a maximal yield of n-hexadecane could be obtained with a minimal excess of LiAlH₄; this latter value determines the radiochemical

TABLE I. Synthesis of *n*-hexadecane by reduction with LiAlH_4 of cetyl iodide, cetyl bromide, sodium *n*-hexadecyl sulfate and *n*-hexadecyl *p*-toluenesulfonate. Reactions were carried out with 25 mmol of the tested compound, dissolved or suspended in 100 ml of solvent (Et_2O = diethyl ether; THF = tetrahydrofuran). Yields were determined by GLC; for the sulfate and tosylate, cetyl bromide was used as internal standard.

	Molar ratio product : LiAlH_4 (theor. 1:0.25)	Time of reflux (hours)	Solvent used	Yield %
Cetyl iodide	1 : 0.28	6	Et_2O	37
	: 0.28	24	Et_2O	42
	: 0.28	9	THF	45
	: 0.50	24	THF	75
	: 0.50	48	THF	76
	: 0.75	24	THF	97
	: 1.00	24	THF	100
Cetyl bromide	1 : 0.28	15	THF	48
	: 0.33	6	THF	62
	: 0.75	24	THF	95
	: 1.00	24	THF	100
Sodium <i>n</i> -hexadecyl sulfate	1 : 0.28	24	THF	2
	: 0.50	24	THF	39
	: 0.75	24	THF	69
	: 1.00	24	THF	100
<i>n</i> -Hexadecyl <i>p</i> -toluenesulfonate	1 : 0.28	24	THF	13
	: 0.50	24	THF	51
	: 0.75	24	THF	88
	: 1.00	24	THF	100

yield of the reaction. Since low yields were obtained in ether, most of the reactions were carried out in refluxing tetrahydrofuran (Table I). Optimal chemical yields were obtained with a molar ratio of 1:0.75 for cetyl bromide and cetyl iodide, and with a molar ratio of 1:1.00 for sodium *n*-hexadecyl sulfate and tosylate (no *n*-hexadecanol was formed from these compounds). Therefore, cetyl bromide or cetyl iodide should be used for reduction with tritiated LiAlH_4 . Since no significant differences between both products were noted when treated with a

three-fold excess of LiAlH_4 , cetyl bromide, which is less costly, purer and more stable than cetyl iodide, was chosen for reaction with LiAlT_4 .

For the synthesis of n-hexadecane-1-T, approximately 5.0 mCi of LiAlT_4 (specific activity 237 mCi/mmol; New England Nuclear, Dreieichenhain, West-Germany) was added to a suspension of 711 mg of LiAlH_4 (18.75 mmol) in 100 ml of dry THF and refluxed under nitrogen with 7.63 g of cetyl bromide (25.0 mmol) for 26 h. Excess reagent was decomposed with water and the precipitated hydroxides were dissolved in 100 ml of 2N H_2SO_4 . The aqueous solution was extracted three times with ether; the combined organic layers were washed with brine and dried over anhydrous Na_2SO_4 . GLC analysis of the ether extract showed a complete conversion into n-hexadecane. After evaporation of the solvent, the residue of n-hexadecane-1-T (7 ml) was diluted with 159 ml of n-hexadecane and distilled in vacuum to give 159 ml (2225 μCi) of n-hexadecane-1-T with a specific activity of 14.0 $\mu\text{Ci}/\text{ml}$.

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