SYNTHESIS OF HIGH SPECIFIC ACTIVITY TRITIUM-LABELLED DOTRIACONTANE

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Received October 28, 1976
Revised December 29, 1976

SUMMARY

The tobacco smoke particulate phase marker dotriacontane was labelled with tritium at positions 15, 16, 17 and 18 by the catalytic reduction of dotriaconta-15, 17-diyne. This was obtained by oxidatively coupling hexadec-1-yne, in turn prepared by reaction of monosodium acetylide with myristyl bromide.

Key Words: Dotriacontane, Tritium, Catalytic Reduction, Tobacco Smoke

INTRODUCTION

Studies of retention of tobacco smoke particulate phase in the respiratory system of smoke-exposed laboratory animals are facilitated by inclusion of a suitable particulate phase marker compound in the tobacco. Earlier studies at these[1-3] and other laboratories[4,5] have utilised ^{14}C-labelled dotriacontane and unlabelled decachlorobiphenyl to determine total smoke dose. More recently, however, high resolution autoradiography has been used to study particulate phase localisation within the respiratory tract[6]. For this type of study it was essential to use tritium labelled dotriacontane having a specific activity of approximately 0.5 Ci/mmol. The tritiation procedure described in the ensuing text should, however, be suitable for labelling at a much higher level than warranted by our own needs.

Submission of a sample of purified dotriacontane to the Wilzbach labelling procedure[7] at The Radiochemical Centre resulted, after repeated column chromatographic purification and recrystallisation, in material of unacceptably low specific activity. It was concluded, therefore, that reduction of a

0362-4803/78/0214-0163$01.00/0

suitable unsaturated precursor using tritium gas might well be the most appropriate means of introducing non-labile tritium atoms into the molecule. This paper describes the synthesis of the diacetylene dotriaconta-15,17-diyne and its reduction to dotriacontane-15,15,16,16,17,17,18,18-^{3}H.

Higher acetylenes may be conveniently prepared by reaction of monosodium acetylide with the appropriate alkyl halide; oxidative coupling then yielding the diacetylene. In the reaction sequence outlined below sodium acetylide, prepared by bubbling acetylene through a solution of sodium in liquid ammonia, is reacted with myristyl bromide in dimethyl formamide to give hexadec-1-yne (I). Purified I is oxidatively coupled using cupric acetate in pyridine:methanol to yield dotraiconta-15,17-diyne (II). The diyne is reduced to the alkane (III) using tritium gas in the presence of Adams catalyst:

$$CH \equiv CH \xrightarrow[\text{liq.}NH_3]{Na} CH \equiv CNa \xrightarrow[\text{DMF}]{\text{myristyl bromide}} \underset{\text{(I)}}{CH_3\,(CH_2)_{13}C = CH} \;\; + NaBr$$

$$2x(I) \xrightarrow[\text{MeOH/pyr/Et}_2\text{O}]{\text{CuOAc}} \underset{\text{(II)}}{CH_3(CH_2)_{13} - C \equiv C\text{-}C \equiv C\text{-}(CH_2)_{13}CH_3}$$

$$II \xrightarrow[\text{dioxan}]{\overset{*}{H}_2/Pt} \underset{\text{(III)}}{CH_3(CH_2)_{13}(CH^*_2)_4(CH_3)_{13}CH_3}$$

EXPERIMENTAL

Hexadec-1-yne. To liquid ammonia (1ℓ) cooled in solid CO_2/acetone was added ferric nitrate (0.1 g) as a catalyst[8] followed by sodium metal (36.6 g, 1.59 mol) added slowly over a period of 1-2 hr[9]. Acetylene, from which traces of acetone had been removed by passage through a trap cooled in solid CO_2/ acetone, was passed through the above sodamide solution for approximately 4 hr and then the ammonia was allowed to evaporate (CAUTION: on no account should liquid nitrogen be used as coolant for the acetone traps in view of the explosive nature of solid or liquid acetylene (B.pt.-84°C)). Myristyl bromide (173.2g, 0.625 mol) in dimethyl formamide (600 ml) was added and the mixture was carefully heated under nitrogen to 65°C and maintained at that temperature for 3 hr. The mixture was cooled, poured into distilled water/ice and extracted with hexane (3 x 250 ml). The combined extracts were washed successively with water, dilute hydrochloric acid, water, sodium bicarbonate solution and saturated sodium chloride solution before being dried over anhydrous sodium sulphate. Removal of the hexane by rotary film evaporation yielded a mobile reddish-brown oil having infra-red absorption maxima at 3310 cm^{-1} and 2110 cm^{-1} [10]. The oil was fractionated at reduced pressure using a Vigreux column and a main fraction (55 g) collected at 110-111°C/0.15mm; nd^{23} = 1.4416 (lit value[11] = 83-91°C/0.07mm; nd^{20} = 1.4420). Found C,86.0; H,13.6% (calc for $C_{16}H_{30}$: C, 86.4,H,13.6%).

Dotriaconta-15,17-diyne. Anhydrous cupric acetate was first prepared by heating cupric acetate monohydrate under reflux with acetic anhydride for 2 hr. The solid was cooled, filtered off, washed with anhydrous diethyl ether and dried in vacuo.

Hexadec-1-yne (5.5 g, 0.025 mol) and anhydrous cupric acetate (10 g) were heated under reflux on a steam bath for 4 hr with pyridine (330 ml) and anhydrous diethyl ether (1 l). The course of the reaction was followed by submitting portions of reaction mixture to gas-liquid chromatography on a glass column packed with SE30 on Chromosorb G operating at 300°C. The diethyl ether was removed by distillation and the reaction mixture poured into water and extracted three times with diethyl ether (3 x 250 ml). The aqueous layer was discarded and the ether layer washed successively with water, dilute hydrochloric acid, water and finally saturated sodium chloride solution before being dried over anhydrous sodium sulphate. Removal of solvent by rotary film evaporation afforded an oil which slowly crystallised from ethyl acetate and was isolated as small platelets (3.2 g) m.p. = 47°C. Found C,86.9;H,12.8% (calc for $C_{32}H_{58}$:C,86.8;H,13.2%). A molecular weight of 442.8 was confirmed by mass spectrometry.

Dotriacontane. Conditions for quantitative reduction of dotriaconta-15,17-diyne were first established using hydrogen. A closed system incorporating a gas burette was used in order to measure the volume of hydrogen consumed during the reaction. A suspension of Adams catalyst (0.05 g) in methanol was magnetically stirred for 2 hr at room temperature in an atmosphere of hydrogen. A solution of dotriaconta-15,17-diyne (0.2 g, 0.45 mmol) in dry dioxan (50 ml) was added dropwise and reduction allowed to proceed until uptake of hydrogen ceased. (Expected uptake of hydrogen at NTP = 44.8 ml; observed uptake = 40.0 ml). The catalyst was carefully filtered off using Kieselguhr filter aid and solvent removed by evaporation, leaving a white solid (0.2g) which was re-crystallised from diethyl ether and isolated as small white platelets (0.15g, mp 69.7°C). Found C,85.1;H,14.5% (calc for $C_{32}H_{66}$:C,85.3;H,14.8%). A molecular weight of 450.8 and a chemical purity of > 99% (measured against authentic standard) were confirmed by mass spectrometry and gas-liquid chromatography respectively.

Reduction of dotriaconta-15,17-diyne (0.5g, 1.13 mmol) using gaseous tritium (5Ci) was carried out at The Radiochemical Centre, Amersham, using conditions essentially as describe above (Tritium Labelling Service, Code TR3).

Purification of Reduction Product. The total mass of crude dotriacontaine-15,15,16,16,17,17,18,18-^{3}H received from The Radiochemical Centre (1.02 Ci; 95.8 mg) was carrier diluted with unlabelled dotriacontane (404.3 mg) and the mixture repeatedly re-crystallised from diethyl ether to constant specific radioactivity. The product (342 mCi; 314.8 mg) was submitted to gas chromatography using conditions described previously and found to contain 97.4% dotriacontane and 2.6% dotriaconta-15,17-diyne. Autoradiographic examination of a sample submitted to thin layer chromatography on silica gel using 4:1 cyclohexane:ethyl acetate indicated a single spot containing all of the detectable radioactivity. From these data the specific radioactivity of purified dotriacontane-15,15,16,16,17,17,18,18-^{3}H was calculated to be 0.50 Ci/mmol.

ACKNOWLEDGEMENT

The authors thank the Tobacco Research Council for permission to publish this work. Mass spectra were produced by Dr. B. Blessington, University of Bradford and microanalytical data by F.B. Strauss Laboratories, Oxford.

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