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NOTES

A SIMPLE APPARATUS FOR REDUCTIVE LABELLING WITH ISOTOPIC HYDROGEN GAS AT ATMOSPHERIC PRESSURE

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SUMMARY

A simple apparatus is described for the generation of isotopic hydrogen gas by acid-catalysed hydrolysis of sodium borotritide or borodeuteride. The apparatus may be conveniently utilised for isotopic hydrogen reductions of from 20 μmol to 1 mmol scale.

Key Words: hydrogen tritide, hydrogen deuteride, tritium-labelling, nedocromil sodium, Tilade^{R}, microhydrogenator.

INTRODUCTION

Catalytic reduction with isotopic hydrogen gas is one of the most commonly used procedures for labelling organic compounds with tritium or deuterium (1,2). Of the six isotopomeric forms of hydrogen gas only dihydrogen, dideuterium and ditritium are commercially available. Hydrogen deuteride, hydrogen tritide and deuterium tritide are not available and must be prepared as required. Of these isotopomers hydrogen tritide is of particular interest since, in the absence of rapid isotopic scrambling, alkene reduction with this isotopomer yields tritium-labelled compounds in which only a single labelled atom is introduced into the molecule. The radiochemical stability of the resulting labelled compound is therefore maximised whilst differences between the marker and unlabelled compound due to isotope effects are minimised. Moreover, hydrogenations with ditritium rarely utilise quantities less than 1 Ci and are usually carried out at near

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maximum specific radioactivity. For most metabolic applications and many other tracer studies quantities of radioactivity and specific radioactivities up to three orders of magnitude smaller than the above are desired. To meet these requirements a small scale hydrogenation apparatus (Figure 1) has been constructed, which allows the generation of hydrogen tritide, or other desired isotopomers, by the hydrolysis of labelled sodium borohydrides (3). The hydrogenator is simple to use and easily constructed from commonly available high performance liquid chromatography equipment. Examples of the use of the apparatus to prepare ^{3}H- and ^{2}H-isotopomers of the pharmaceutical agent (4) nedocromil sodium 1 by reduction of allyl derivatives are also described.

DISCUSSION

Description of the apparatus

The apparatus is shown diagramatically in Figure 1. It is constructed from stainless steel high performance liquid chromatography equipment which is connected to pyrex glassware via glass to metal seals. A vacuum pump is connected to the apparatus at A and the vacuum attained may be read from the vacuum gauge (C). A two-way stainless steel valve (D) is used to isolate the apparatus from the vacuum as desired. Hydrogenation flasks (Q) or vials (M) may be attached to the stainless steel tee (G) via glass to metal sealed B10 cone(N), or via a stainless steel adaptor (J) and a greased 'O'-ring (K), respectively. Hydrogen tritide access to the hydrogenation flask or vial is through the right hand channel of a stainless steel two-stem three-way valve (L) which is connected to the hydrogen tritide reservoir (S) via a glass to metal seal. The reservoir consists of an inverted burette containing a solution of acetic acid in carbon tetrachloride (P). Hydrogen tritide is generated in the reservoir, when a weakly alkaline solution of sodium boro[^{3}H]hydride (H) is injected through the left hand channel of valve (L) into the burette via the septum (F). This septum consists of a short length of silicone rubber capillary tubing which closely fits the needle of the gas-tight injection syringe.

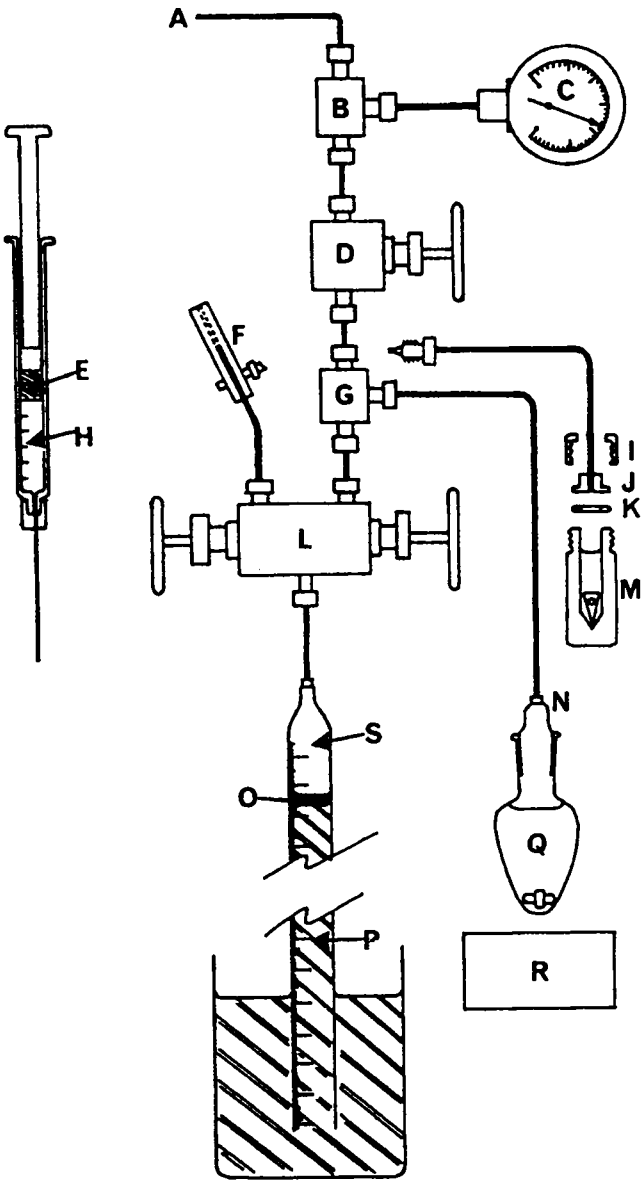


Figure 1: Diagram of the microhydrogenation apparatus

A, Outlet to vacuum pump. B, Tee connector (1/16"). C, Vacuum gauge. D, Two-way valve. E, Hydrogenation solvent. F, Silicone rubber capillary tube. G, Tee connector (1/16"). H, Alkaline borohydride solution. I, Screw cap. J, Stainless steel adaptor. K, Greased 'O' ring. L, Dual-stem three-way valve. M, Vial and flea. N, Glass to metal seal (2 mm i.d.). O, Hydrogenation or wash solvent. P, Burette of from 1.0 to 50 cm^3 capacity containing carbon tetrachloride plus the appropriate acetic acid. Q, B10 flask and flea. R, magnetic stirrer. S, Isotopic hydrogen.

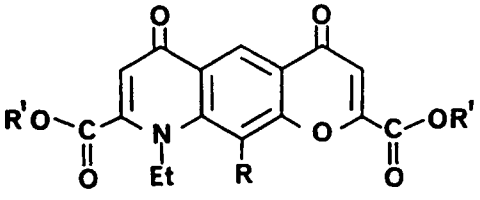
Use of the apparatus

The apparatus may be used for hydrogenations with H_2, D_2, HD or with H_2/HT or HT/DT mixtures by suitable selection of the borohydride and acetic acid isotopomers used. Detailed examples of the use of the apparatus are given in the Experimental section. Generally the course of hydrogenation reactions is best monitored by observing the rate of hydrogen uptake. If quantitative uptake studies are required, corrections to the observed gas volume consumed must be performed. These include corrections for temperature, ambient pressure, hydrostatic pressure changes, catalyst absorption and solvent vapour pressure. Provided that the corrections are made the apparatus will deliver good accuracy.

EXPERIMENTAL

Component suppliers

Stainless steel SSI valves, were obtained from Anachem Ltd, Luton, Beds, UK. Glass tubing of 2 mm internal diameter sealed to 1/16" steel tubing was used for construction of the burette and B10 cone glass to metal seals and was obtained from JJ's (Chromatography) Ltd, Kings Lynn, Norfolk, UK. Thick-walled screw-cap vials of nominal capacity of 1.0 cm^3 were used as hydrogenation vials and were obtained from Pierce (UK) Ltd, Cambridge, Cambs, UK.



1. R = $CH_2CH_2CH_3$, R' = Na
2. R = $CH_2CH{=}CH_2$, R' = Na
3. R = $CH_2CH{=}CH_2$, R' = Et
4. R = $CH_2CH_2^*CH_3^*$, R' = Et
5. R = $CH_2CH_2^*CH_3^*$, R' = Na

Figure 2

Reagents

Disodium 9-ethyl-6,9-dihydro-4,6-dioxo-10-prop-2-enyl-4H-pyrano[3,2-g]quinoline-2,8-dicarboxylate 2 and the corresponding diethyl ester, 3 were obtained from Fisons plc, Pharmaceutical Division, Loughborough, UK. Sodium boro[^{3}H]hydride at a specific radioactivity of 3.5 GBq mmol^{-1} (95 mCi mmol^{-1}) was obtained from Commissariat a l'Energie Atomique, Centre d'Etudes Nuclèaires de Saclay, Gif-sur-Yvette, France. Sodium boro[^{2}H]hydride was obtained from Stohler Isotope Chemicals, Waltham, Ma 02154, USA. Deuterium oxide, [O-^{2}H]ethanoic acid and [O-^{2}H]methanol were obtained from the Aldrich Chemical Company Ltd, Gillingham, Dorset, UK. Sodium [^{2}H]hydroxide (40 weight % in deuterium oxide) was obtained from the Sigma Chemical Company Ltd, Poole, Dorset, UK. All other reagents were of reagent grade.

Reduction with hydrogen tritide: preparation of [2,3-^{3}H]nedocromil sodium

Sodium boro[^{3}H]hydride (nominal 25 mCi, 95 mCi mmol^{-1}) was dissolved in potassium hydroxide solution (0.5% by weight, 0.5 cm^3) and the majority of the solution drawn into a gas-tight syringe of 1 cm^3 capacity. Ethyl acetate (0.2 cm^3) was also drawn into the syringe to flush the valve L (Figure 1) clear of borohydride during the injection process. The burette was completely filled with a mixture of carbon tetrachloride and glacial acetic acid (9:1 by volume) and the syringe contents injected via the septum F. After standing for five minutes 22.8 cm^3 of gas had been evolved; equivalent, after correction for temperature, hydrostatic, atmospheric and solvent vapour pressure, to 20.05 mCi of hydrogen tritide. The flask Q was charged with diethyl 9-ethyl-6,9-dihydro-4,6-dioxo-10-prop-2-enyl-4H-pyrano[3,2-g]quinoline-2,8-dicarboxylate, 3 (435 mg), 5% rhodium on carbon catalyst (50 mg) and ethyl acetate (18 cm^3) and stirred for five minutes prior to the careful application of vacuum via valve D. The contents of the flask were allowed to boil for two minutes after which valve D was closed and hydrogenation initiated by opening the right hand channel of valve L. Uptake of hydrogen tritide was followed until the burette was empty of gas, at which point helium gas was introduced into the burette via the septum F

from a cylinder fitted with a syringe needle outlet. Uptake of the residual hydrogen tritide in the flask was subsequently monitored by briefly opening the right hand channel of L at intervals. When uptake had ceased the flask and burette were emptied of gas by re-application of vacuum and the reaction completed with unlabelled hydrogen, introduced into the burette as for helium. This procedure ensured the absence of unreacted precursor which was known to label under the experimental conditions via an exchange process (5). After filtration and crystallisation from hexane/ethyl acetate, diethyl 9-ethyl-6,9-dihydro-4,6-dioxo-10-[2,3-^{3}H]propyl-4H-pyrano[3,2-g] quinoline-2,8-dicarboxylate, 4(H*=^{3}H) (12.2 mCi) was obtained. The material had a specific radioactivity of 28.7 μCi mg^{-1} and a radiochemical purity >99%. ^{3}H-nmr (96MHz,C^{2}HCl$_3$) showed ^{3}H resonances for the 2 and 3 positions only. The tritium isotope abundance at position 3 was twice as great as at position 2, reflecting methylene group exchange prior to reduction (5).
To the above ester was added 11 cm^3 of a solution of sodium hydroxide (0.193 mol dm^{-3}) in ethanol/water (77:23 by volume) and the resulting solution refluxed for eighty minutes. After cooling acetone (30 cm^3) was added and the crude nedocromil sodium allowed to crystallise overnight. Subsequently, the clear supernatant was decanted, the residue dissolved in water (2 cm^3) and the pH adjusted to 6.5 with glacial acetic acid. Acetone (20 cm^3) was then added to initiate crystallisation and after standing for three hours the crystals were filtered, washed with acetone (10 cm^3) and dried under vacuum to yield disodium 9-ethyl-6,9-dihydro-4,6-dioxo-10-[2,3-^{3}H]propyl-4H-pyrano[3,2-g]quinoline-2,8-dicarboxylate, [2,3-^{3}H]nedocromil sodium, 5 (H* = ^{3}H), 5.64 mCi, 24.6 μCi mg^{-1}. The material was identical with authentic nedocromil sodium by ^{1}H-nmr (360 MHz, $^{2}H_2O$) and by thin layer chromatography in eight solvent systems. The radiochemical purity of the material was >98.6% in the same eight systems.

Reduction with dideuterium: preparation of [2,3-^{2}H]nedocromil sodium

Disodium 9-ethyl-6,9-dihydro-4,6-dioxo-10-prop-2-enyl-4H-pyrano[3,2-g]quinoline-2,8-dicarboxylate, 2, (200 mg) was dissolved in

[O-^{2}H]methanol (12 cm^3) and the solution adjusted to pH 9 with triethylamine. Rhodium on carbon catalyst (50 mg) was added and the stirred suspension hydrogenated with excess dideuterium gas. The dideuterium was prepared by injecting sodium boro[$^2H_4$]hydride (11 mg) dissolved in 0.5 cm^3 of a solution of sodium [^{2}H]hydroxide (0.5% in deuterium oxide) into a solution of [O-^{2}H]acetic acid in carbon tetrachloride (1:10 by volume) via the septum F (Figure 1). When uptake of the dideuterium gas was complete (one hour) the suspension was filtered, the pH adjusted to 6.5 with glacial acetic acid and the solvent removed under reduced pressure. The resulting solid was azeotroped once with water (5 cm^3) and twice with methanol (7.5 cm^3) and the residue dissolved in water (0.5 cm^3). The product was induced to crystallise by the addition of ethanol (4.5 cm^3) and acetone (10 cm^3). Filtration yielded [2,3-^{2}H]nedocromil sodium 5 (H* = ^{2}H) (161 mg). ^{1}H-nmr, δ (360 MHz, ^2H_2O), 8.75 (1H, s, 5-H), 6.95 (1H, s, 7-H), 6.45 (1H, s, 3H), 4.50 (2H, q, NCH_2CH_3), 3.25 (2H, d, $ArCH_2CH_2CH_3$), 1.75 (1.24H, m, Ar $CH_2CH_2CH_3$), 1.1 (3H, t, NCH_2CH_3), 0.95 (0.84H, m, $ArCH_2CH_2CH_3$) ppm; ^{2}H-nmr, δ (55 MHz, H_2O), 1.70 and 0.90 broad singlet resonances ratio 1:2.77.

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