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A Simple Preparation of Hexadeuteriocholesterol

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SUMMARY

26,26,26,27,27,27 Hexadeuterio cholesterol has been prepared in 9 steps from pregnenolone and hexadeuterio acetone with an overall yield of 7 %.
Key Words: Cholesterol-d_6

INTRODUCTION

Due to the physiological importance of cholesterol and its role in the development of cardiovascular diseases, much effort has been devoted to the determination of its absorption, metabolism and excretion (1). Considerable use has been made of isotopically labelled compounds, especially those containing carbon and/or hydrogen isotopes. The introduction of 6 deuteriums in the side chain has been used recently in two major contributions in this field (2,3). The standard procedure for the quantitative assessment of the pruducts for example in studies of their absorption and excretion has been GC-Ms using single ion monitoring (SIM). By the simultaneous observation of a number of characteristic fragments and by a considerable amount of computation it is possible to assess the relative concentrations of normal and labelled material in a sample. The procedure represents a major improvement

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compared with earlier methods in which use was made of labelling of cholesterol with radioactive carbon and hydrogen isotopes.

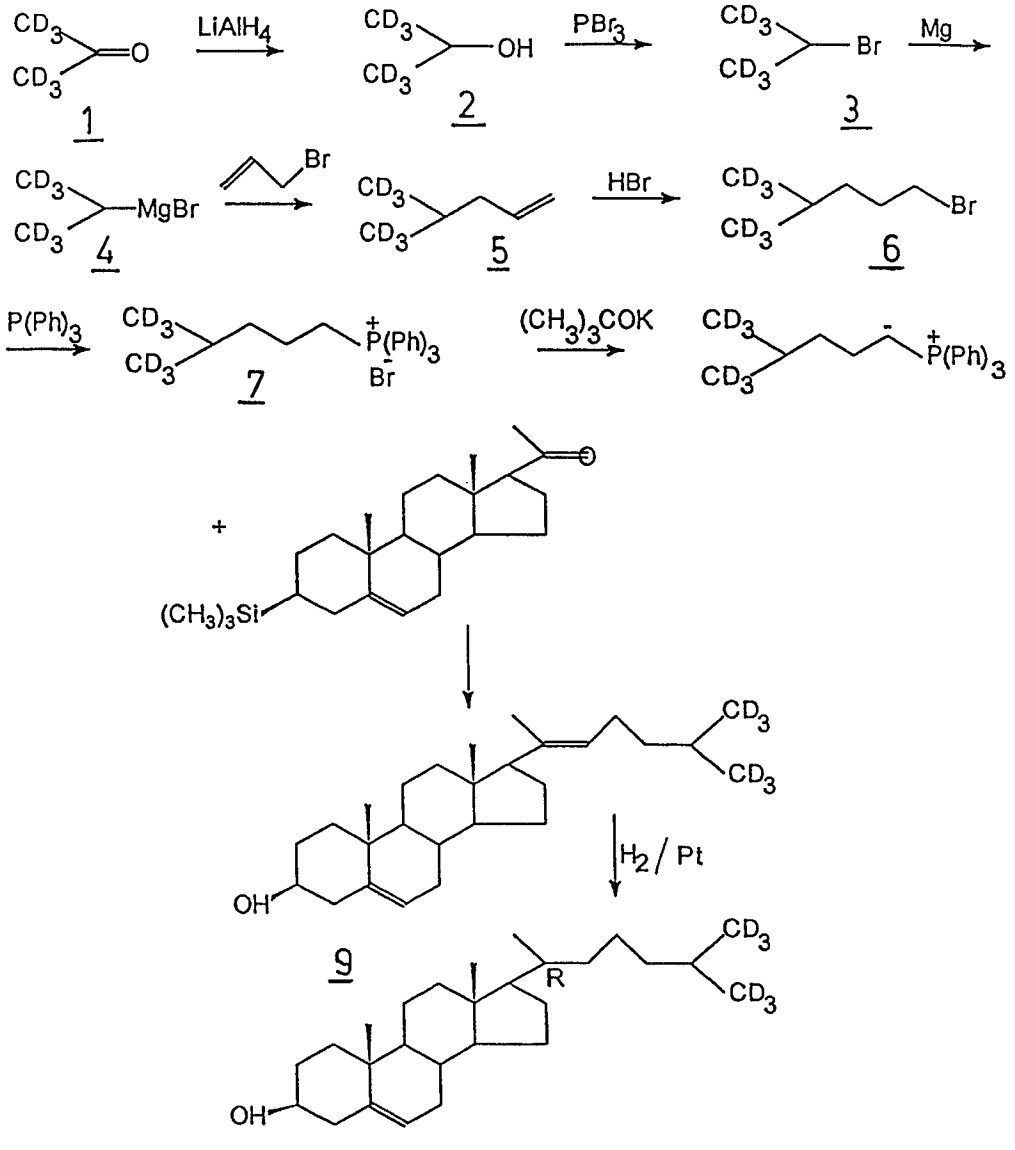
A disadvantage of the new method is the high cost of the required cholesterol multisubstituted with deuterium or ^{13}C. The only published synthesis of cholesterol with 6 deuteriums in the terminal methyl groups of the side chain uses the inaccessible and very costly desmosterol as starting material (4).

Because of the usefulness of the compound a simple procedure for its preparation is needed, which is based on easily available and cheap starting materials. We here report a 9 step synthesis starting with inexpensive perdeuterio acetone and pregnenolone and leading to 26,26,26,27,27,27-^{2}H cholesterol in an overall yield of 7 %.

RESULTS AND DISCUSSION

The key step in the synthesis (Scheme 1) is the condensation of d_6-isopropylmagnesium bromide with allyl bromide to form d_6-4-methylpentene (5). An anti-Markovnikov addition of HBr yields d_6 4-methylpentylbromide (6) which reacts with triphenylphosphine to form the phosphonium salt (7).The formation of the Wittig reagent and its reaction with pregnenolone to form Δ-20-cholesterol and the reduction to cholesterol has been described (5,6). A problem was that a 5-7 times excess of the Wittig reagent is used. When the hydroxy group was protected by silylation the excess of the Wittig reagent could be reduced to 3.5 times with a corresponding improvement of the yield. The catalytic reduction of the double bond was performed as described using platinum oxide at atmospheric pressure (5,6).

SCHEME 1



Hexadeuteriocholesterol

EXPERIMENTAL

d_6-isopropanol (2). To 250 ml of diethyl carbitol (diethylene glycol diethyl ether) was added 17 g $LiAlH_4$ with stirring under nitrogen. A solution of 60 g of d_6-acetone (0.938 M) in 250 ml diethyl carbitol was added dropwise. Fractionation through a glass helices column yielded 46.7 g d_6-isopropanol (75 %) b.p. 76-78 °C.

d_6-isopropyl bromide (3). To the alcohol in an ampoule was added slowly with cooling and shaking 64.8 g of phosphorous tribromide . The sealed ampoule was kept 3 days at room temperature. The contents were washed with water, with dilute potassium carbonate and again with water. After drying with magnesium sulphate distillation through the column produced 70.5 g (76 %) of product b.p. 56-58 °C.

d_6-4-methyl-1-pentene (5). 70.5 g (0.54 M) of the bromide was dissolved in 500 ml of dry dibutylether and reacted with 13 g of sublimed magnesium in a sealed 1 l ampoule with magnetic stirring. To the Grignard reagent was added over 1 hour with stirring a solution of 60.5 g (0.5 M) allyl bromide in 200 ml of dibutylether; 0.1 g portions of cuprous bromide were added every 20 min. A heavy phase, containing magnesium bromide, separated. Stirring was continued for a further 1 hour. 500 ml of water was added cautiously and the ether layer separated, dried and distilled. Below 130 °C some 60 g were collected. Redistillation yielded a fraction b.p 50-60 °C (45 g) which according to ^{1}H NMR contained 25 % allyl bromide. 14 g of diethylamine was added and after 16 h a crystal mass had formed. The product was washed with cold water and dilute hydrogen chloride, dried and distilled: 30 g (62 %) bp. 52-58 °C.

d_6-4-methylpentyl bromide (6). To the methylpentene was added 0.1 g of benzoylperoxide. Hydrogen bromide was added at 0 °C

over the course of 3 h. The product was washed with water, dried and distilled. 40 g were collected b.p. 144-48 °C (71 %).

d_6-4-methylpentyltriphenylphosphonium bromide (7). 38.6 g (0.226 M) of the hexylbromide was mixed with 59.1 g of triphenylphosphine and heated at 80 °C overnight. The crystals were triturated with benzene, the product filtered, washed with benzene and dried to yield 75 g (77 %), of the required salt m.p. 220-232 °C.

d_6-20-dehydrocholesterolacetate (9). 1.5 g (3.5 mMol) of the phosphonium salt was reacted with 0.4 g potassium-tert-butylate in 5 ml of benzene in a sealed ampoule heated on the steam bath with shaking for 2 hours. A dark brown solution of low viscosity was obtained. 350 mg (0.91 mMol) of pregnenolone silylated with trimethylsilane was added and the sealed ampoule was heated on the steam bath overnight. The product was taken up in water and ether. The ether phase was washed with 2N HCl and with $NaHCO_3$. The contents of the ether phase were separated by thin-layer chromatography (Kiesel-gel, 20 % ethyl acetate/pentane, 3 plates) to yield 230 mg of the free hydrol, which was acetylated with 1 ml each of pyridine and acetic anhydride overnight. The mixture, when poured on ice and dilute HCl yielded crystals which were filtered, washed with water, and dried. 234 mg (0.61 mMol, 67 %). ^{1}H NMR 250 MHz (δ,$CDCl_3$): 5.38 (1H,d H_6), 5.17 (1H,t H_3), 4.60 (1H,d H_{17}), 2.05 (3H,s CH_3CO), 1.63 (3H,s H_{21}), 1.02 (3H,s H_{19}), 2.0 - 1.0 (25H,m).

REFERENCES

1. Grundy, S.M. Absorption and metabolism of dietary cholesterol. Annu. Rev. Nutr. 3 71.

2. Lutjohann, D., Meese, C.O., Crouse, J.R. and von Bergmann, K. - J. Lip. Res. 34: 1039 (1993).
3. Bosner, M.S., Ostlund, R.E. Jr., Osofisan, O., Grosklos, J., Fritschle, C. and Lange, L.G. - J. Lip. Res. 34: 1047 (1993).
4. Kirk, D.N. - J. Chem. Soc. Perkin Trans. 1: 2563 (1983).
5. Schmit, J.P., Piraux, M. and Pilette, J.F. - J. Org. Chem. 40: 40 (1586).
6. Schow, J.P. and McMorris, T.C. - J. Org. Chem. 44: 3760 (1979).