SYNTHESIS OF THE EPIMERIC 2-BROMOCHOLESTEROLS AND THEIR CONVERSION INTO [2α-^{3}H]CHOLESTEROL AND [2ß-^{3}H]CHOLESTEROL

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SUMMARY: The synthesis of the epimeric 2-bromocholesterols is described. Their reductive catalyzed debromination with T_2 affords [2α-^{3}H]- and [2ß-^{3}H]cholesterol respectively. The [2-^{3}H]cholesterols can be synthesised with a radiochemical purity >98% and specific activities of about 600 - 700 GBq/mmol. The labelling reactions proceed under retention.

KEY WORDS: Synthesis, 2-bromocholesterols, tritium labelling, reductive catalyzed debromination, tritium labelled cholesterol

INTRODUCTION

For the synthesis of [2α-^{3}H]- and [2ß-^{3}H]cholesterol the corresponding 2-bromocholesterols should be useful starting materials. However, these compounds are not described in the literature. In 1964 Collins and Hobbs had reported [1] that on reduction of 6ß-bromocholest-4-en-3-one 1a cholesterol 2a resulted in high yield instead of the expected 6ß-bromocholest-4-en-3ß-ol 3a . We used this reaction for the preparation of [7α-^{3}H]cholesterol from 6ß-bromo-[7α-^{3}H]cholest-4-en-3-one [2,3].

Now we tried to apply this method for the synthesis of the epimeric 2-bromocholesterols 2b,2c which should be converted into tritium labelled cholesterols [4] by the known halogen-tritium exchange. In the present work the successful syntheses of [2α-^{3}H]cholesterol 2d and [2ß-^{3}H]cholesterol 2e with high specific activities are described.

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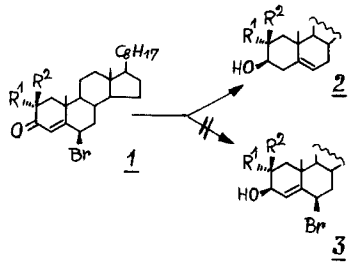

	R^1	R^2
a	H	H
b	Br	H
c	H	Br
d	T	H
e	H	T

RESULTS and DISCUSSION

The $NaBH_4$ reduction of 2α,6ß-dibromocholest-4-en-3-one 1b [5,6] in bis[2,2'-dimethoxyethyl]ether (diglyme) gave a colourless oil consisting of two main components according to thin-layer chromatography (TLC). The products were separated by column chromatography on silica gel. The first compound eluated (25 - 30 %) was 2α-bromocholest-5-en-3α-ol as shown by ^{13}C-NMR spectroscopy [7]. The main product (70 - 75 %) was the desired 2α-bromocholesterol 2b [7].

For preparation of 2ß-bromocholesterol 2c, 2ß,6ß-dibromocholest-4-en-3-one 1c was no useful starting material because of its instability. Therefore 2c was effectively prepared by $NaBH_4$ reduction in diglyme of the more stable 2ß-bromocholest-5-en-3-one synthesised in high purity by a procedure of Ellis and Petrow [5] and characterized by its ^{13}C-NMR spectrum [8]. According to TLC the obtained white solid consisted of two substances with hardly differing R_f values. After column chromatography on silica gel they were obtained in a pure state. The first product eluated (about 8 %) was 2α-bromocholesterol 2b formed by a side reaction (inversion of the 2ß-bromo-substituent due to the alkaline medium). It was followed by the desired 2ß-bromocholesterol 2c with a yield of 90 %.

In order to characterize 2b and 2c elemental analyses, IR spectra and mass spectra were used. The mass spectroscopy showed the peak of the molecular ion M^+ at m/e 464,405 (calc. 464,402 for $C_{27}H_{45}OBr$) and revealed the fragments $M-CH_3$, M-Br, M-HBr, $M-Br-H_2O$, and $M-C_8H_{17}$. The structures were fully confirmed by ^{13}C-NMR spectroscopy.

The chemical shifts of the ring A and ring B carbon atoms of 2a, 2b, and 2c are listed in Table 1. The assignments of the signals of 2b and 2c were made by comparison with the spectra of 2a [9] and 2α-bromocholestan-3ß-ol [9]. The substituent effects arising from 2α-Br or 2ß-Br are in agreement with data known from other bromosteroids [8,9,10]. Further explanations are given in [7].

The reactivity of the 2-bromocholesterols corresponded with the determined configuration. Whereas 2b was stable to alkali because of its equatorial bromine, 2c having an axial bromine was easily dehydrobrominated to the enol 4 which was converted via 5, finally, into the stable cholest-4-en-3-one 6. The conversion was quantitative allowing to determine the content of 2c in mixtures with 2b by potentiometric bromide titration.

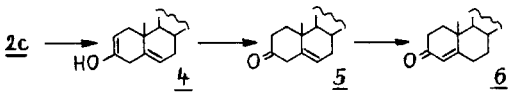

Table 1 : ^{13}C chemical shifts of C(1) - C(10) and C(19) in cholesterol 2a, 2α-bromocholesterol 2b and 2ß-bromocholesterol 2c

carbon atom	chemical shifts [a] 2a [b]	2b	2c
1	37,3	48,5	45,1
2	31,6	59,8	60,9
3	71,6	76,3	71,8
4	42,2	39,8	37,9
5	140,6	138,5	139,9
6	121,4	123,2	122,8
7	31,9	31,6	31,2
8	31,9	31,8	31,7
9	50,2	49,9	51,4
10	36,5	40,3	37,2
19	19,4	19,7	22,8

a) in ppm relative to TMS

b) see reference [9]

The reductive debromination of 2b with H_2 was possible only in a rather strong alkaline solution of 0.13 N NaOH in a mixture of dioxane and ethanol with an excess of Pd catalyst in a rather long reaction time. Even under these conditions quantitative conversion into 2a could not be achieved.

In contrast, the reductive debromination of 2c was a rapid reaction in alkaline dioxane in the low concentration range between 0.01 - 0.03 N NaOH. However, the reaction did also not proceed quantitatively and excessive catalyst was necessary for high yields of 2a . At >0.03 N NaOH dehydrobromination was dominating.

Surprisingly, the reductive debromination of 2b with T_2 under the same conditions described above was quantitative and faster than in the inactive case. The calculated amount of tritium gas was taken up. [2α-^{3}H]Cholesterol 2d was isolated by preparative TLC with a radiochemical purity >98% and a specific activity of 685 GBq/mmol .

The reductive debromination of 2c with T_2 needed only a few minutes. The tritium gas consumption was lower than calculated. In comparison with the inactive case we did not find any difference in the reaction rate. Preparative TLC afforded [2β-^{3}H]-cholesterol 2e with a radiochemical purity >98% and a specific activity of 580 GBq/mmol .

The radio TLC results of the reductive catalyzed debromination of 2b and 2c with T_2 are summarized in Table 2 . In both cases the arrangement of the peaks was similar. However, in case of debromination of 2c the intensity of peak 1 was lower and the intensity of peak 3 directly depended on the alkali concentration of the reaction medium. At 0.02 N NaOH peak 3 represented 26.2 %, at 0.03 N NaOH 65 % of the activity. On the other hand, the amount of [2β-^{3}H]cholesterol 2e (peak 2) diminished from 30 % to 18.8 % . Isolation of peak 3 by preparative TLC and identification afforded [4α,5α-^{3}H]cholestan-3-one 7 formed from 6 . The reaction 6→7 means that the more alkali is present the more 2c is lost for 2c→2e . In order to identify 7 it was converted into [4α,5α-^{3}H]-

cholestan-3ß-ol by $NaBH_4$ reduction and, furthermore, it was brominated to 2α-bromo-[4α,5α-^{3}H]cholestan-3-one and 2,2-dibromo-[4α,5α-^{3}H]cholestan-3-one.

The nature of peak 1 was not further investigated. It can be assumed to be a mixture of diols according to spot colouring and low R_f value. Manifold development of the radiochromatogram showed several polar substances. The more alkaline the reaction medium the more diols occur as demonstrated by both debromination reactions of 2b and 2c .

As can be seen from the percentages of activity the maxima of 2d and 2e were about 30 % . However, the preparation of 2d was a simpler procedure because of the high sensitivity of 2c against alkaline medium as mentioned above. After preparative chromatography the mean yield of the new tritium labelled cholesterols 2d and 2e was 10 - 15 % .

Under the reaction conditions used here the hydrogenation of the 5-double bond could not be excluded. In the active case we would obtain 2d or 2e accompanied by threefold labelled

Table 2 : Radio TLC results of reductive catalyzed debromination of 2b and 2c with T_2

peak	R_f-value a)	colour of spots b)	activity (%) 2α-Br→2α-T c)	2ß-Br→2ß-T d)	remarks
1	0.03	blue	53.5	25.1	mixture of products; probably diols e)
2	0.11	purple	29.7	30.0	pure 2d or 2e f)
3	0.37	yellow	7.6	26.2	pure 7 f)
4	0.63	-	3.4	3.2	not further investigated
5	0.80	-	5.8	6.2	not further investigated

a) benzene/ether (19 : 1) as solvent
b) after spraying with vanilline sulphuric acid and heating
c) 0.13 N NaOH in dioxane/EtOH (4 : 1)
d) 0.02 N NaOH in dioxane
e) shown by manifold development in Benzene/acetone (9 : 1)
f) shown by chemical reactions (see text)

5α-cholestan-3β-ol. As cholesterol and 5α-cholestan-3β-ol don't differ chromatographically in the TL-systems used here a high specific activity of the cholesterol would be simulated. However, both cholesterols 2d and 2e were shown to be quantitatively converted into the corresponding 5α,6β-dibromo-[2 - ^{3}H]cholestan-3β-ol. This means tritium labelled cholestan-3β-ol was absent or, in other words, the reaction conditions fully avoided the hydrogenation of the 5-double bond.

The configuration of the 2 - ^{3}H of cholesterol was elucidated by addition and substitution reactions. After adding inactive cholesterol the labelled cholesterols were converted into 2α,5α,6β - and 2β,5α,6β - tribromocholestan-3-one as well as into 2α - bromo- and 2,2 - dibromocholestan-3-one. Following the loss of activity there is no doubt that the reductive debromination reactions have proceeded under retention.

MATERIALS and METHODS

The reductive catalyzed debromination reactions with tritium gas (product of USSR) were carried out in a labelling apparatus of the tritium laboratory of the Institute of Nuclear Research Rossendorf [11]. Specific activities were determined in an LS-233 scintillation spectrometer (Beckman, USA). The activity on radiochromatograms was detected by a scanner LB 2723 (Berthold-Friesecke, FRG). Preparative radio TLC was carried out on 20 cm x 20 cm plates (silica gel, 2 mm, Merck). Analytical radio TLC on 15 cm x 15 cm silufol plates (Kavalier, CSSR) was used to determine the activity distribution and the radiochemical purity of purified products respectively. Solvents for chromatography were

solvent 1 ..... benzene/ether (19 : 1),
solvent 2 ..... benzene/acetone (9 : 1).

EXPERIMENTAL

<u>$NaBH_4$ reduction of 2α,6β-dibromocholest-4-en-3-one 1b</u>. 1b [5,6] (2 g = 3.7 mmol) was dissolved in diglyme (freshly dist., 30 ml) by warming and treated under stirring with a suspension of $NaBH_4$ (1 g) in diglyme (5 ml) at r.t. 20 minutes. Water

(50 ml) and benzene (35 ml) were given into the reaction mixture. Under stirring aqueous HOAc (1 : 3) was added dropwise and when the first violent reaction ceased more aqueous HOAc (about 50 ml) was poured into the mixture. The benzenic extract was washed twice with warmed water, dried, evaporated under reduced pressure and the remaining colourless oil was separated by column chromatography using solvent 2. The first product was 2α-bromocholest-5-en-3α-ol (276 mg = 0.6 mmol = 16.2 %). Analysis: Calcd. for $C_{27}H_{45}OBr$ C 69.6, H 9.7, Br 17.2. Found C 68.9, H 10.4, Br 17.4. IR. 672, 680, 805, 828, 885, 960, 1030, 1210, 1385, 1470, 1650, 3500 cm^{-1}. ^{13}C-NMR see [7]. The second product eluated was pure 2α-bromocholesterol 2b (796 mg = 1.7 mmol = 46 %). IR. 607, 700, 760, 805, 828, 930, 960, 990, 1033, 1075, 1160, 1210, 1265, 1380, 1470, 1638, and 3440 cm^{-1}. ^{13}C-NMR see Table 1.

[2α-^{3}H]cholesterol 2d.

2b (55 mg = 118 μmol) was debrominated 2 h in a solvent mixture of dioxane (5 ml), EtOH (abs., 1.5 ml), and 1 N NaOH (1 ml) in presence of Pd black (150 mg) and T_2. The excess of T_2 was reabsorbed, the reaction mixture filtered, the filtrate lyophilized and the residue separated on a Merck plate in solvent 2. After extracting with benzene/ether (1 : 1) 2d was obtained (7.2 mg = 18.7 μmol = 16 %) with a radiochemical purity >98 %. The specific activity was 685 GBq/mmol.

$NaBH_4$ reduction of 2β-bromocholest-5-en-3-one.

2β-bromocholest-5-en-3-one [6] (1.56 g = 3.37 mmol) was suspended in diglyme (freshly dist., 40 ml). Under stirring a suspension of $NaBH_4$ (1 g) in diglyme (5 ml) was added at r.t. forming a colourless solution which was worked up as described above. Column chromatography using solvent 1 afforded as first product a little 2b (52 mg = 0.112 mmol = 3.3 %) and then pure 2c (576 mg = 1.24 mmol = 36.8 %). Analysis: Found C 69.8, H 10.0, Br 17.0. ^{13}C-NMR see Table 1.

[2β-^{3}H]cholesterol 2e

2c (15.3 mg = 33 μmol) was debrominated 10 minutes in a solvent mixture of dioxane (5.5 ml), EtOH (abs., 0.3 ml), and 0.5 N NaOH (0.3 ml) in presence of Pd black (7.5 mg) and T_2. After reabsor-

bing excessive T_2, filtering and lyophilizing the residue was separated on a Merck plate in solvent 1. By extracting with benzene/ether (1 : 1) 2e (1.4 mg = 3.7 µmol = 11.2 %) was obtained with a radiochemical purity >98 % and a sp. act. of 580 GBq/mmol.

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