SYNTHESIS OF LIPOIDAL DRUGS: - Glycerides of radiolabelled aspirin

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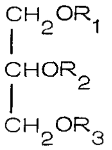
SUMMARY

Triglycerides containing aspirin in place of one or more fatty acid residues have been synthesised. Glycerol, mono and di palmitic acid glycerides were acylated with the acid chloride of /^{14}C-carboxyl/-aspirin to give the corresponding tri-, di and mono-aspirin glycerides.

Key words: Synthesis, Carbon-14, Tritium, Mono, di and tri aspirin glycerides

INTRODUCTION

The present paper describes the preparation of radiolabelled glycerides of aspirin (2-acetoxybenzoic acid) where one, two or all three of the fatty acids in a triglyceride have been replaced by the drug as indicated in Figure I.



CH_2OR_1 | $CHOR_2$ | CH_2OR_3

where R_1, R_2 and R_3 each is hydrogen or fatty acyl and at least one of the groups is:-

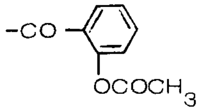


Figure I.

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The object of these preparations was to incorporate water soluble drugs into lipid structures, whereby they would be rendered fat soluble. The mode of metabolism of these 'lipoidal drugs' was significantly different from that of the original drugs. These compounds were not absorbed from the stomach but from the intestine under an alkaline pH and hence ulceration of the stomach which is commonly observed with the use of aspirin was avoided. The detailed studies on the metabolism of these drugs (1,2) warranted the preparation of single (^{14}C) and double radiolabelled (^{14}C and ^{3}H) compounds, the syntheses of which are reported here.

Palmitoyl chloride was reacted with 1-benzyl-glycerol to give 1-benzyl-2,3-dipalmitoyl glycerol which was hydrogenated in the presence of palladium-on-charcoal to give 2,3-dipalmitin 2 (3). The latter on re-acylation with aspirin acid chloride labelled with ^{14}C on the carboxyl, gave the required aspirin containing triglyceride (3 Fig II).

The second aspirin triglyceride analogue, 1,3-dipalmitoyl-2(2'-acetoxy-/¯ ^{14}C-carboxyl_/-benzoyl) glycerol 6 was obtained by the acylation of 1,3-dipalmitin 5 with 2-acetoxy-/¯ ^{14}C-carboxyl_/-benzoyl chloride in chloroform and pyridine (Fig II).

The diaspirin and triaspirin glycerides 7 and 8 were prepared by the direct acylation of monopalmitin 4 , and glycerol 1 with the radiolabelled aspirin acid chloride. The compound 8 was previously described by Ciampa et al (4) as a solid m.p. 81 - 89°C. We were unable to obtain this as a solid, although our product was an oil which gave a single spot on thin layer chromatography (TLC) and analysed correctly for the required compound.

The ^{3}H-labelled compound 5A was also prepared from /¯2(n)-^{3}H_/ glycerol and acylated with aspirin acid chloride to give the 1,3-dipalmitoyl-2(2'-acetoxy benzoyl) /¯2(n)^{3}H_/glycerol 6A . The ^{3}H-labelled compound 6A could be mixed in any proportion with the ^{14}C-labelled compound 6 to give the intermolecularly doublylabelled 1,3-dipalmitoyl-2(2'-acetoxy-/¯ ^{14}C-carboxyl_/ -benzoyl) /¯2(n)^{3}H_/ glycerol for metabolic studies.

In reactions involving the acylation of a number of amines with aspirin chloride, Jenson and Linholt (5) have shown that even after working up the reaction mixture under mild conditions, the resulting acyl compound

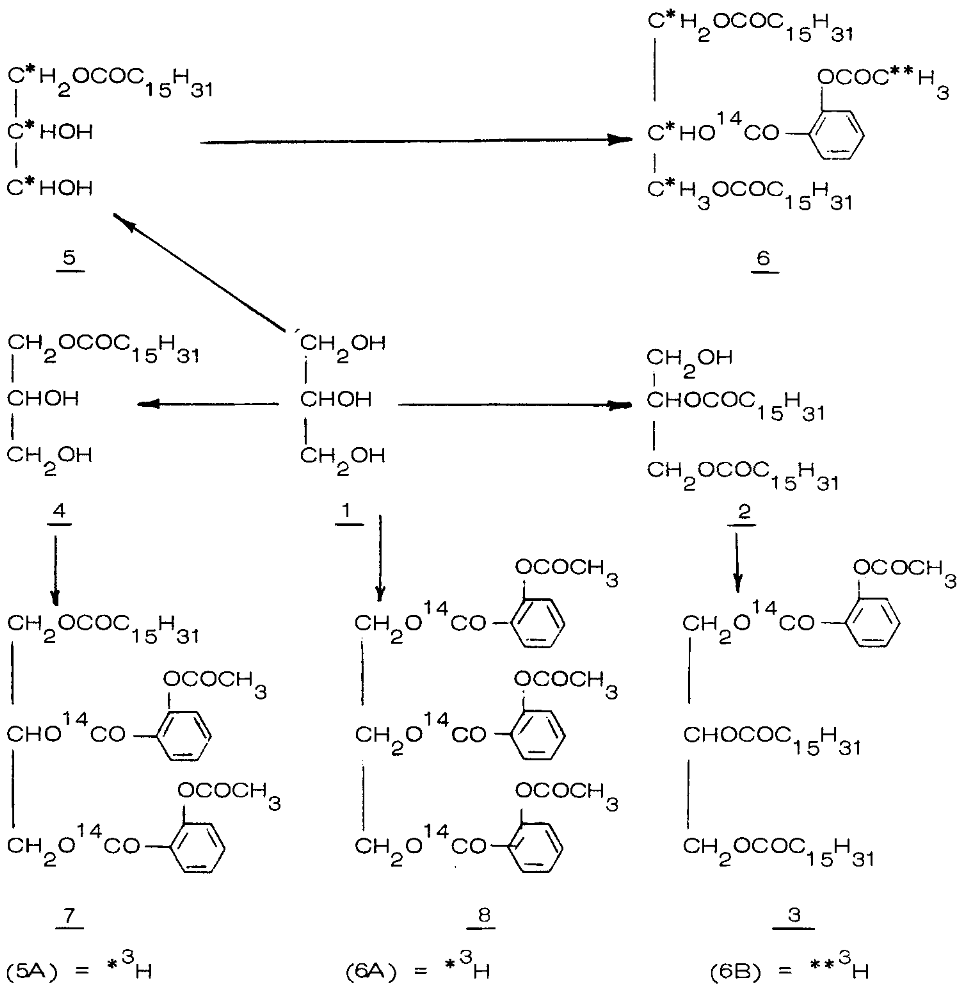

Figure II

contained only the salicylyl instead of the required acetyl salicylyl derivative. In order to confirm that such de-acetylation had not occurred during the synthesis of compound 6, 1,3-dipalmitin was acylated with doubly labelled aspirin acid chloride containing a known ratio of ^{3}H/^{14}C. The compound 1,3-dipalmitoyl 2(2'-[^{3}H]-acetoxy-[^{14}C-carboxyl]-benzoyl) glycerol 6B synthesised contained a ^{3}H/^{14}C ratio which was identical to that in the original di-labelled aspirin acid chloride, showing that de-acetylation had not occurred during synthesis.

DISCUSSION

The preparation of a number of naphthylacetic acid derivatives of glycerol have been recorded in a Syntex patent (6). However, the procedures for preparation of the compounds, especially the 2-substituted glycerides is different from the present procedure. We found the preparation of our 2-aspirin glyceride <u>6</u> via the 1,3-benzylidine glycerol, followed by removing the protecting benzylidine acetal and re-esterification unsuitable as it resulted in unresolvable mixtures on removal of the protecting group, by the method suggested in the patent.

A preparation of 1,2-diacetone-3-(2'-acetoxy-benzoyl) glycerol is given in a Schering Corporation patent (7). Here, the procedure involves a completely different route of synthesis. Although we were easily able to obtain the same aspirin-ketal quite simply by the esterification of diacetone glycerol with aspirin acid chloride, however, on removing the acetone under various hydrolytic conditions we were unable to obtain a pure 1-(2'-acetoxy benzoyl) glycerol).

As far as we are aware, the above patents, our own patent (8) and the already mentioned work of Ciampa et al (4) is the only literature available on these types of compounds. Furthermore, these radio isotopically labelled compounds have not previously been synthesised.

EXPERIMENTAL

Melting points are uncorrected. All solvents used were dried and were of reagent grade. Chloroform was dried over phosphorous pentoxide and distilled before use. Acid chlorides were freshly prepared and distilled before use. The chemical purity of the compounds and the reaction progress were checked by thin layer chromatography (TLC) in two separate solvent systems: chloroform : acetone (96 : 4 v/v) ; diethylether : benzene : ethanol : acetic acid (40 : 50 : 2 : 0.2 v/v). Developed zones were visualised in iodine vapour and the radiochemical purity was determined by liquid scintillation counting (Packard Tri-Carb 3380). Elemental analysis of the compound was carried out by Dr. F.B. Strauss, at the microanalytical laboratory at Oxford.

1-(2'-Acetoxy-[^{14}C-carboxyl]-benzoyl)2,3-dipalmitoyl glycerol 3

[^{14}C-carboxyl]acetylsalicylic acid (250 µCi, specific activity, S.A. 22 mCi/mmol, Radiochemical Centre, Amersham, Bucks) was diluted with carrier acid (3 g, 16.65 mmol) and dissolved in dry benzene (20 ml) and refluxed with oxalyl chloride (5 ml) for 2½ hours at 60 - 70°C. The excess oxalyl chloride and solvent were evaporated in vacuum and the distilled acid chloride (1.09 , 5.49 mmol, 82.5 µCi) dissolved in $CHCl_3$ (20 ml) was added dropwise to 2,3-dipalmitin (2.6 g, 4.57 mmol) in $CHCl_3$ (25 ml) and pyridine (2 ml). The mixture was maintained at 0°C during the addition (1 hour) and stirred overnight at room temperature. The mixture was washed successively with 0.5 M HCl (2 x 15 ml), 0.5 M $NaHCO_3$ (2 x 15 ml) and cold water (2 x 20 ml). The organic layer was dried over anhydrous Na_2SO_4, the solvent evaporated to dryness and the product taken up in a small volume of petroleum ether and chromatographed on a silicic acid column (32 g silicic acid in a column of 2.5 cm diameter). A stepwise elution with increasing quantities of ether in petroleum ether (200 ml, 1% v/v; 250 ml, 5% v/v ; 500 ml, 80% v/v; 150 ml, 25% v/v and 500 ml, 100% v/v) was used to elute the product and the required compound was found in the 8% eluate. The eluate was evaporated to dryness. The residue was dissolved in $CHCl_3$ and methanol was added until a turbidity was observed and the compound allowed to crystallise at -20°C. A colourless solid m.p. 52°C (3.02 g, 90% yield, 61.98 µCi, 21.98 mCi/mmol) was obtained. The radiochemical purity was 99.8% as shown by TLC. [Found C, 72.40 ; H, 10.44 ; $C_{44}H_{74}O_8$ (M = 730.99) requires C, 72.29 ; H, 10.20].

1,3-Dipalmitoyl-2(2'-acetoxy-[^{14}C-carboxyl]-benzoyl) glycerol 6

Acetylsalicyloyl chloride obtained from acetylsalicylic acid (1.26 g, 6.3 mmol, 95.45 µCi) was dissolved in chloroform (20 ml) and dripped slowly over one hour into a stirred solution of 1,3-dipalmitin (3.0 g, 5.28 mmol) in chloroform (25 ml) and pyridine (2 ml). The mixture was stirred at 0°C for 2 hours and then overnight at room temperature. The reaction mixture was worked up as described earlier for compound 3. The oil after crystallisation from petroleum ether (40 - 60°C) at 4°C gave colourless crystals of the compound 6 , m.p. 47.5°C (3.45 g, 89% yield based on dipalmitin, S.A. 21.90 mCi/mmol). The radiochemical purity was 99.80% as shown by TLC [Found C, 72.14 ; H, 10.08 ; $C_{44}H_{74}O_8$ (M = 730.99) required C, 72.29; H, 10.20%]

1,3-Dipalmitoyl-[2(n)-^{3}H] glycerol 5A

This compound was prepared by the modified method of Rose (9). [2(n)-^{3}H] Glycerol (5 mCi, Radiochemical Centre, Amersham, Bucks) was mixed with anhydrous glycerol (23.6 mg, 0.256 mmol) pyridine (4 ml) and dry chloroform (10 ml). Palmitoyl chloride (142 mg, 0.516 mmol) in dry chloroform (15 ml) was added to the above stirred solution at 0^{o}C. The reaction mixture was stirred overnight at room temperature, diluted with ether (50 ml) and washed successively with 0.5 M HCl (2 x 50 ml), and 0.5 M $NaHCO_3$ (2 x 50 ml) and finally with cold water (2 x 50 ml) and the organic layer was dried over anhydrous sodium sulphate, evaporated to give a colourless solid. The yield of impure labelled 1,3-dipalmitin was 93.4 mg (63.8%) of radiochemical purity 67.9%. The product was further purified by preparative TLC (using a reference unlabelled 5, silica gel G impregnated with 5% boric acid developed in chloroform, acetone 96 : 4 v/v). Isolation and recrystallisation of the pure compound (58.3 mg, 39.8% yield based on glycerol) gave a radiochemical purity of 99.3%.

1,3-Dipalmitoyl-2(2'-acetoxy benzoyl) -[2(n)-^{3}H] glycerol 6A

The purified 1,3-dipalmitoyl [^{3}H]glycerol 5A was dissolved in chloroform (10 ml) and pyridine (0.5 ml) and reacted with acetylsalicyloyl chloride (61 mg, 0.31 mmol) in chloroform (10 ml), under similar conditions as described before. The compound 6A m.p. 48.5^{o}C was purified by eluting from an alumina column and crystallised from ether and methanol. The purified compound 6A gave a single spot on thin layer chromatography with a radiochemical purity of 99.9%.

1,3-Dipalmitoyl-2 (2' [^{3}H]-acetoxy-[^{14}C-carboxyl]-benzoyl) glycerol 6B

[^{3}H] Acetic anhydride (14.57 g, 142.72 mmol, 992 µCi, S.A. 100 mCi/mmol, Radiochemical Centre, Amersham, Bucks) was added to ^{14}C labelled salicylic acid (10 g, 72.4 mmol, 50 µCi, S.A. 61 mCi/mmol) and the mixture was refluxed with 2 drops of concentrated sulphuric acid for 2 hours at 60^{o}C. The product was cooled in an ice bath and distilled water (150 ml) was added with stirring. The mixture cooled overnight and the precipitate was filtered off. The dry residue was crystallised from anhydrous ether (450 ml) and dry petroleum ether (40 - 60^{o}C, 750 ml) to obtain white needles (10.8 g, 82.73% based on salicylic acid, ^{3}H/^{14}C ratio 6.25, S.A. ^{14}C = 60.7 mCi/mmol)

The acid chloride of 2'-[^{3}H]acetoxy-[^{14}C-carboxyl] benzoic acid (4 g, 22.2 mmol) was dissolved in 10 ml of chloroform and dripped slowly into a stirred solution of 1,3-dipalmitin (2.5, 4.4 mmol) in chloroform (15 ml) and pyridine (0.6 ml, 8.7 mmol). After working up the reaction mixture, the product was crystallised from chloroform/methanol at -20°C. The product was further purified for microanalysis by eluting through the silicic acid column as described for 3 and finally crystallised from petroleum ether (40 - 60°C) at -20°C m.p. 47°C (2.9 g, 90.1%) [Found C, 71.99, H, 9.98, $C_{44}H_{74}O_{8}$ (M = 730.99) requires C, 72.29, H, 10.20, ^{3}H/^{14}C ratio = 6.247, S.A. 60.9 mCi/mmol based on ^{14}C]. The purified compound on TLC gave a single spot and had a radiochemical purity of 99.7% for ^{14}C and 99.80 for ^{3}H.

1-Palmitoyl 2,3-di (2'-acetoxy-[^{14}C-carboxyl]-benzoyl) glycerol 7

[^{14}C]Acetylsalicyloyl chloride (9.53 g, 47.98 mmol, 250 µCi S.A. 22 mCi/mmol) in 15 ml chloroform was dripped into the monopalmitin (6.6 g, 20 mmol) solution in chloroform (15 ml) and stirred for 48 hours at room temperature. After working up the reaction mixture as for 3 the solution was evaporated to a yellow viscous oil which was purified by eluting through a silicic acid column with 15% ether in petroleum ether (40 - 60°C). On evaporating the eluate, it gave the compound 7 as an oil η_{D}^{60} = 1.67315, (11.0 g, 84%, 99% radiochemical purity on TLC). [Found C, 67.68 : H, 7.69, $C_{37}H_{50}O_{10}$ (M = 654.80) requires C, 67.86 ; H, 7.69%].

1,2,3-Tri (2'-acetoxy-[^{14}C-carboxyl]-benzoyl) glycerol 8

[^{14}C] Acetylsalicyloyl chloride (17 g, 85.6 mmol, 250 µCi S.A. 21 mCi/mmol) in anhydrous chloroform (75 ml) was dripped into a stirred solution of dry re-distilled glycerol (2 g, 21.71 mmol) in anhydrous pyridine (6.8 ml) and chloroform (15 ml) kept at 0°C. After stirring overnight at room temperature the reaction mixture was worked up as described for 3 . The solution was evaporated to a yellow viscous oil. The oil was purified by eluting it through a silicic acid column with 60% benzene in hexane. The eluate on evaporation gave an oil η_{D}^{60} = 1.6847 (11.0 g, 87.5%. radiochemical purity 99.84% on TLC.[Found C, 62.61, H, 4.58, $C_{30}H_{26}O_{12}$ (M = 578.54) requires C, 62.28 : H, 4.53%].

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