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Some Selected Abstracts Relevant for Radiosynthesis

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Synthesis of Tritium Labeled Coenzymes and Related Compounds

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Radiolabeled coenzymes of nucleotide origin and their precursors are widely used in biochemical, medical, and molecular biology studies. This communication presents comparative data on the methods of incorporating tritium in related nucleosides, nucleotides and coenzymes via chemical reactions, isotope exchange reactions, followed by enzymatic conversions.

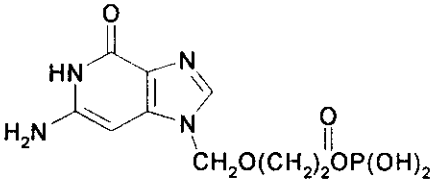
The **catalytic dehalogenation** of various respectively functionalized purines and pyrimidines with tritium gas are reported to give incorporation rates ranging from 3% to 91% of the theoretical values, which, for a given catalyst, proved to be dependent upon the structure of the substrates employed[1,2]. With Pd on various carriers the following specific activities could be achieved: [2-^{3}H]adenine 24 Ci/mmol; [2,8-^{3}H]adenine 48 Ci/mmol; [5-^{3}H]uracil 18 Ci/mmol; [6-^{3}H]uracil 23.8 Ci/mmol; [5,6-^{3}H]uracil 44 Ci/mmol; [5-^{3}H]uridine 13 Ci/mmol.

Moreover, methyl substituted purines/pyrimidines and their corresponding nucleosides can be frequently labelled by **catalytic deoxygenation** of the respective hydroxymethyl or formyl precursors with gaseous tritium. Thus reduction of 5-hydroxymethyl-2'-desoxyuridine, of 5-hydroxymethyluracil, and of 5-formyluracil gave thymine probably preferentially labeled with tritium in the methyl group with the following specific activities (Ci/mMol): 27.5; 25.0; and 55.0 respectively.

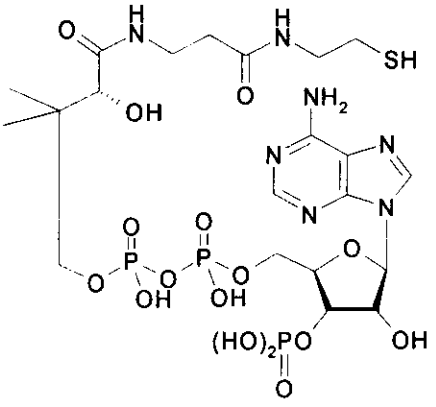
A more general, highly versatile approach to a wide variety of labeled purines/pyrimidines and their respective nucleosides, however, which does not need any halogenated or oxygenated precursors, is offered by **heterogeneous catalytic H/T-exchange procedures** with gaseous tritium either in a solid state or in solution at temperatures between 160 and 200°C [5,6,7,8]. This methodology applied to adenine afforded tritiated material with a specific activity of 54 Ci/mmol; [^{3}H]guanine showed a specific activity of 24.9 Ci/mmol, [^{3}H]xanthine of 25 Ci/mmol, [^{3}H]hypoxanthine of 51 Ci/mmol, [^{3}H]benzyladenine of 180 Ci/mmol, [^{3}H]furfuryladenine of 160 Ci/mmol, [^{3}H]adenosine of 120 Ci/mmol, [^{3}H]acyclovir of 125 Ci/mmol, and [^{3}H]acyclovir phosphate **1** of 56 Ci/mmol. Finally, even several oligonucletides such as Poly-A(7,8s), Poly-U(12s), and RNA 15s could be tritiated at 180°C giving specific activities of 10.2 Ci/g., 12.0 Ci/g., and 12.1 Ci/g.

Enzymatic methods have proved as a powerful tool to convert the abovementioned labeled purines/pyrimidines into their corresponding nucleosides/nucleotides with the aid of individual **enzymes, poly-enzymatic mixtures**, as well as of **whole cells**. Thus by optimization of the respective reaction conditions nucleotides with different degrees of phosphorylation (e.g.: [^{3}H]ADP, [^{3}H]ATP) become preparative conveniently available by using an E.coli poly-enzymatic preparation. Furthermore, [^{3}H]NAD selectively labeled in the adenine moiety was obtained with the help of an enzyme from Sacharomyces cerevisiae (nicotineamide nucleotide adenilyl transferase).

Although the solid state catalytic exchange reaction with gaseous tritium could be successfully applied to label several nucleotides at specific activities > 10 Ci/mmol it failed, however, in the case of [^{3}H]Coenzyme A ([^{3}H]CoA) **2**.



1



2

In spite of extensive optimization investigations on various catalysts and temperatures the specific activities achieved were in the range of only 1-4 Ci/mmol. Therefore the described stepwise approach was followed. Divergent, however, from the procedure described so far the label was introduced in the pantothenic acid moiety by the the solid state isotopic exchange on barium pantothenate with gaseous tritium at 170°C. The resulting tritiated intermediate (52 Ci/mmol, 11% yield) was then converted to the final product in the presence of *bredibacterium ammoniagenes cells* as follows: D-pantothenic acid (Ba salt, 100 mCi, spec.act.: 52 Ci/mmol) was dissolved in 125 ml 0.3 M potassium phosphate buffer solution pH 6.5 to which adenosine triphosphate(100 mMol), L-cysteine(25 mMol), and magnesium sulphate (0.1 mMol) were added bringing the total volume to 250 ml. The resulting solution was incubated for 24 hrs at 37 °C with 25 mg of acetone powder of *bredibacterium ammoniagenes* and the final product was isolated by preparative HPLC yielding 30 mCi of [^{3}H]Coenzyme A with a specific activity of 32 Ci/mmol.

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Preparation and Use of Tritiated Silanes

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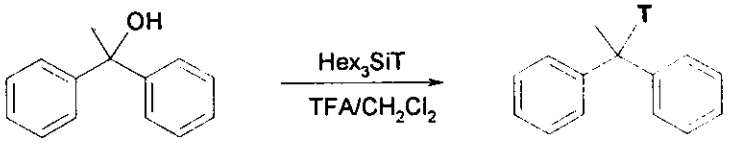
*(present adress: Pharmacia and Upjohn, Uppsala, Sweden)

Several tritiated reducing agents, such as boranes and stannanes, have previously been prepared from LiT which is formed by reacting T_2 gas with n-BuLi in the presence of TMEDA.

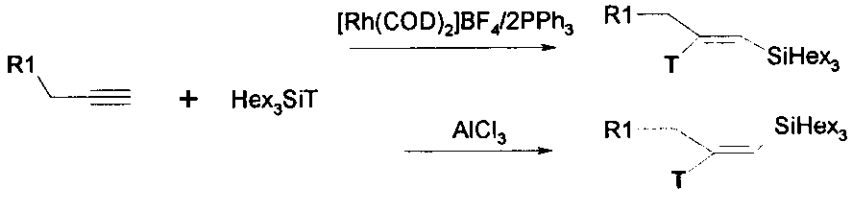
R = Me, Et, Hex, TMS, Ph,

A new area to explore was the preparation of tritiated silanes, as well as the scope and limitations of their applications. The tritiated silanes are prepared by adding the corresponding chlorosilane to LiT. The tritiated alkyl silanes were used in ionic hydrogenations, stereoselective additions to triple bonds and in vinyl triflate reductions. In addition tritiated tris(trimethylsilyl)silane was prepared and used in radically induced deoxygenation reactions as well as radical additions to triple bonds.

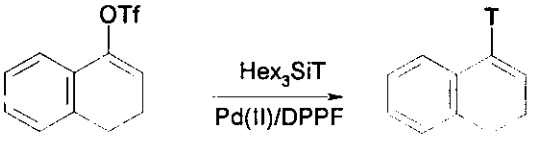
Ionic hydrogenation, using alkyl silane in presence of trifluoroacetic acid, offers a mild method for reducing benzylic alcohols to the corresponding alkanes.



Alkyl silanes add readily to triple bonds in high stereoselectivity in the presence of $[Rh(COD)_2]BF_4/2PPh_3$ for E-alkene and $AlCl_3$ for Z alkene as catalysts.

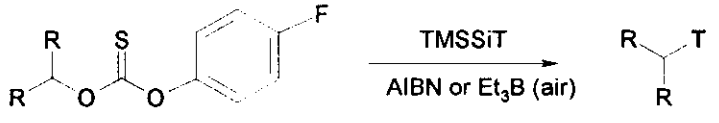


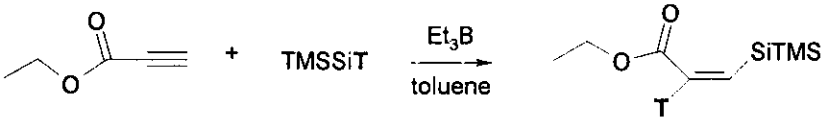
Vinyl triflates are reduced by alkyl silanes with Pd (II) as catalyst.



The most commonly used alkyl silane is triethylsilane and therefore the corresponding tritiated silane was prepared. Since some of these reactions did not work well in THF, the preparation of triethylsilyltritide was prepared in high boiling triglyme, from which controlled distillation of the silane was possible. However, some remaining TMEDA from the preparation of LiT codistilled together with the silane. TMEDA poisoned the transmetalation catalysts in the triflate reduction and the addition reactions. The tritide of choice proved to be trihexylsilyltritide which is very suitable for labelling. The trihexylsilyltritide is high boiling and can be conviently purified by filtration over silica using hexane with good retention of the starting chlorosilane, LiCl, and traces of TMEDA. The trihexylsilyltritide was prepared in up to 97 % yield with a tritium content of more than 98 %.

Tributyltinhydride is the most frequently used compound for radical reactions, but several phenylated silanes have also been used for this purpose. However, the superior silane for radical reactions is tris(trimethylsilyl)silane (TMSSiH). We have prepared TMSSiT in 92 % yield and have used it in radical deoxygenations and radical additions to alkynes initiated by AIBN, Et_3B/O_2.





INCORPORATION OF TRITIUM AND CARBON-14 IN THE SKELETON OF PHARMACOLOGICALLY INTERESTING GESTAGENIC STEROIDS

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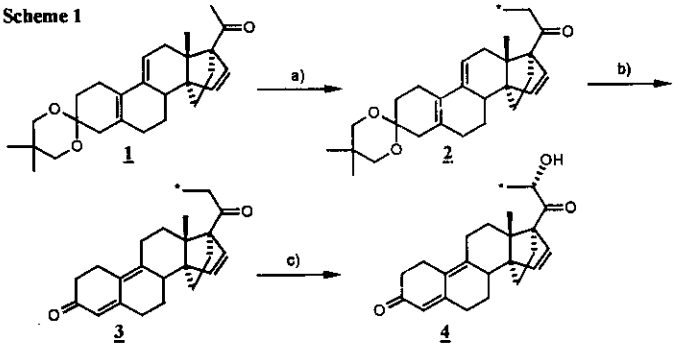
Introduction

Estrogens are used together with gestagens as combination contraceptive agents. Although there are a lot of gestagenic compounds on the market, there is still a need for potent compounds in order to reduce the dose of endogenous hormones and unwanted side effects. Here, the ^{3}H- and the ^{14}C-labelling syntheses of the new gestagens **4** and **9** are described.

Experimental

Starting from the non-active precursor **1** the ^{14}C-label was introduced by alkylation with [^{14}C]methyl iodide to give **2** in reasonable yields. After removal of the ketal protection the 21-hydroxy group was introduced stereoselectively by using a suspension of yeast fungi in nutrient broth. Finally the crude product was purified by HPLC to afford **4** (Scheme1).

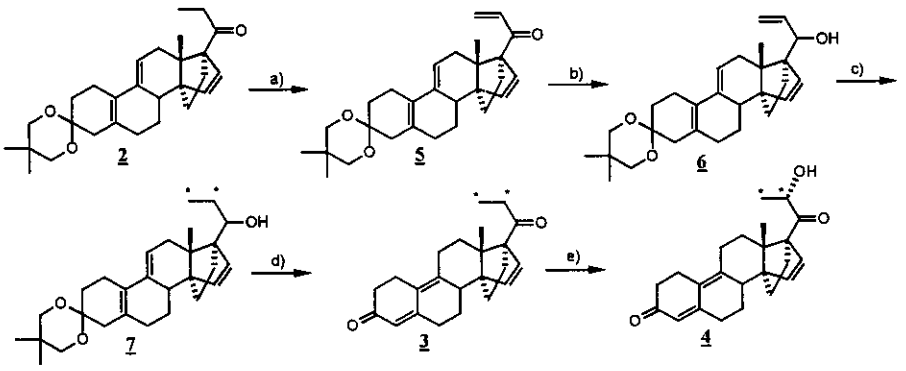
Scheme 1



a) LDA / [^{14}C]MeI, b) HOAc/$HClO_4$, c) Yeast Fungi

Unfortunately all attempts to establish the tritium label in the same way failed. Therefore another double bond was created in the side chain (Scheme 2). After conversion to the allylic alcohol (**6**) a selective reaction with tritium gas / rhodium catalyst could be achieved (**7**). After oxidation of the alcohol the synthetic pathway was completed as described for the ^{14}C-label.

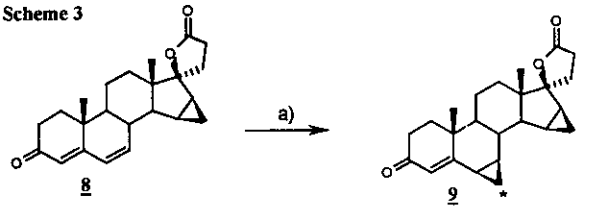
Scheme 2



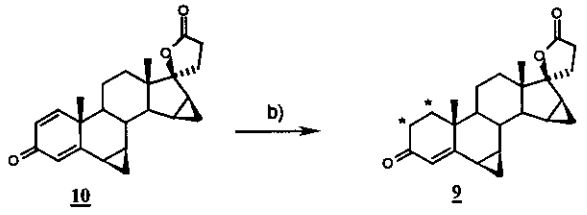
a) 1. LDA / -78°C / PhSeCl - 2. MCPBA / -30°C, b) $NaBH_4$ / $CeCl_3$, c) $[H]H_2$ / $RhCl[P(Ph_3)_3]$ / toluene / RT,
d) 1. PCC - 2. $HOAc/HClO_4$, e) Yeast Fungi

The labelling syntheses of the gestagen **9** were both accomplished by single step reactions (Scheme 3). Reaction of **8** with trimethylsulfonium iodide afforded the 5ß,6ß-cyclopropane derivative **9** as major product. The tritiation was carried out via reduction of the Δ1 double bond (**10**) without effecting Δ4.

Scheme 3



a) Trimethylsulfonium iodide / NaH



a) $[H]H_2$ / $RhCl[P(Ph_3)_3]$ / toluene/EtOH 1:1

The synthesis and analysis of some isotopically labelled 11β-arylsteroids

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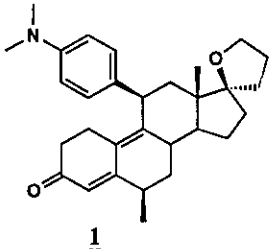
Introduction

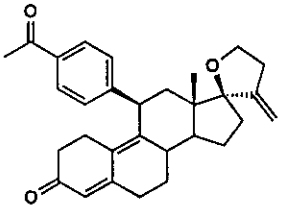
The 11β-arylsteroids Org 31710 (1), Org 33628 (2), Org 34517 (3) and Org 34850 (4) show varying extends of antiprogestagenic and antiglucocorticoid activities.
At various stages of development these compounds were required in isotopically labelled forms. The compounds labelled with stable isotopes were required as internal standards for GC-MS and LC-MS assays. The compounds labelled with different radio-isotopes were prepared for binding experiments and various metabolism studies.

Results and Discussion

[N-C^2H_3]-Org 31710 was prepared by methylation of mono-desmethyl-Org 31710 with C^2H_3I in acetonitrile. [20,22-$^3H_4$]-Org 31710 was prepared by a catalytic reduction of a propargylic precursor with $^3H_2$-Pd/$BaSO_4$ in ethyl acetate for 5 hours. Subsequent hydrolysis and ring closure gave the aimed [20,22-$^3H_4$]-Org 31710 with a remarkably low specific activity of 15 Ci/mmol in 40 % overall yield. ^{3}H-NMR analysis indicated 6 % of the label at C-23 as a result of allylic/propargylic exchange and 1 % of the label at C-11 as a result of benzylic exchange.



1



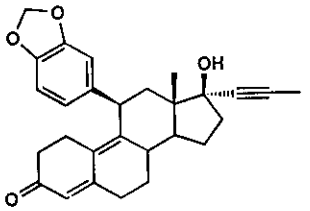
2

Both [21-$^{13}C^2H_2$,22-$^2H_2$]-Org 33628 and [21-$^{14}CH_2$]-Org 33628 were prepared by a Wittig reaction of a 20-keto precursor followed by hydrolysis and dehydration.

[23-$^{13}C^2H_3$]-Org 34517 was synthesized by the alkylation of a lithiated acetylenic precursor with $^{13}C^2H_3I$ in tetrahydrofuran at room temperature and subsequent hydrolysis and dehydration. ^{2}H-NMR analysis revealed the presence of 2-3 % of [23-$^{12}C^2H_3$]-Org 34517.

[23-$^{14}CH_3$]-Org 34517 was prepared in a similar manner, except that the alkylation was done at -78 °C → 20 °C. The values for the specific activity of the final product as determined by both PBEI-MS (44 mCi/mmol) and ^{13}C-NMR (48 mCi/mmol) were in sharp contrast with the value for the specific activity of the $^{14}CH_3I$ (28 mCi/mmol) used as starting material.

Also [23-C^3H_3]-Org 34517 was prepared in a similar manner. ^{3}H-NMR analysis revealed two labelled species, probably [23-C^3H_3]-Org 34517 and [23-$C^1H_1{}^3H_2$]-Org 34517. The calculated specific activity from this assumption (80 Ci/mmol) corresponded very well with the specific activity determined by LC-MS.



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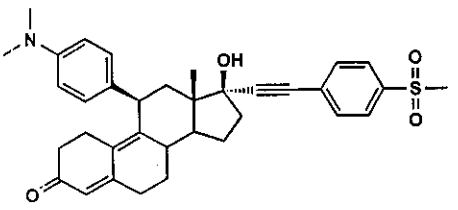

4

[N(C^2H_3)$_2$]-Org 34850 was prepared by the methylation of 4-bromoaniline with C^2H_3I, followed by conversion into a cuprate, conjugate addition to a vinylic epoxide, Heck coupling with 4-bromo-phenyl methyl sulfone, hydrolysis and dehydration.

The synthesis of [methanesulfonyl-^{14}C]-Org 34850 started with the alkylation of 4-bromothiophenol with $^{14}CH_3I$. The intermediate [methyl-^{14}C]-4-bromothioanisole was oxidized to the corresponding 4-bromo-phenyl [^{14}C]-methyl sulfone with oxone. Heck coupling of this sulfone with an acetylenic precursor and subsequent hydrolysis and dehydration gave the aimed [methanesulfonyl-^{14}C]-Org 34850. The values for specific activity (54-57 mCi/mmol) of the final product as determined by FAB-MS, ^{1}H-NMR and ^{13}C-NMR corresponded well with the value for the specific activity of the $^{14}CH_3I$ (57 mCi/mmol) used as starting material.

Tritiated Org 34850 was prepared by catalytic reduction of 4-bromo-3-iodophenyl methyl sulfone with $^3H_2$-Pd/C in ethanol for 16 hours at room temperature. Heck coupling of the [3-^{3}H]-4-bromo-phenyl methyl sulfone with the acetylenic precursor and subsequent hydrolysis/dehydration gave [^{3}H]-Org 34850 with a specific activity of 15 Ci/mmol.

Methods for Mineralization of Carbon-14 Labelled Waste into Barium [^{14}C] carbonate

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Summary

A method for the mineralization of organic matter by anodic oxidation in a silver sulfate containing sulfuric-chromic acid was developed. This electrochemical process can also be used for the complete oxidation of difficult organic wastes, such as hydrophobic substances like hydrocarbons, plastics, activated carbon, etc.

Keywords: Anodic oxidation, waste mineralization, sulfuric-chromic acid

Introduction

Highly radioactive carbon-14 labelled organic waste with different chemical properties represent a severe environmental risk. It is necessary to convert this hazardous material, that is partly decomposed by autoradiolysis, into barium carbonate. Barium carbonate is a widely accepted stable chemical form for safe disposal of carbon-14. However, it requires, the complete oxidation of carbon-14 labelled organic matter to carbon dioxide. For this purpose we have examined the following processes for mineralization of non-labelled and labelled model compounds: TiO_2-photocatalyzed mineralization, sulfuric-chromic acid, ammonium peroxydisulfate, hydrogen peroxide with a catalytic mixture of transition metal salts, and mediated anodic oxidation in silver containing sulfuric-chromic acid. The best results were obtained by the mediated anodic oxidation in silver containing sulfuric-chromic acid /1/.

Experimental

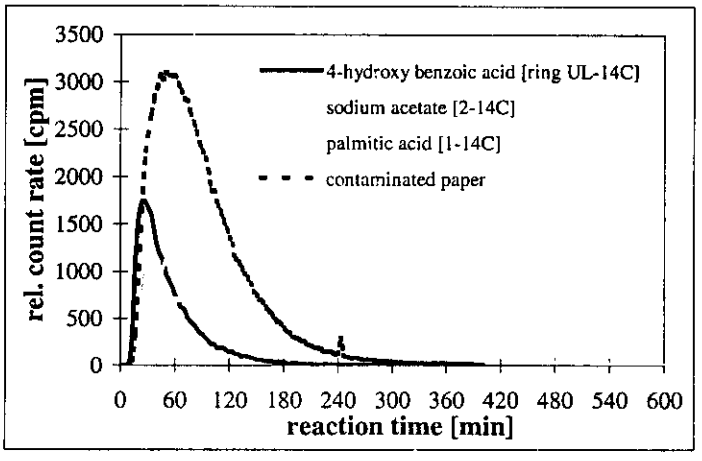
The organic substances were mineralised in a small H-cell that had stationary platinum electrodes separated by a Nafion® 350 cation exchange membrane. The electrochemical cell is charged with an anolyte of 65% sulfuric acid containing chromium trioxide, silver sulfate, and a catholyte of 20 - 40% sulfuric acid. The anodic compartment has a heating jacket and is connected to a cupric oxide catalyst filled quartz tube, and gas traps containing 2M sodium hydroxide. To on-line measure the radioactivity of $^{14}CO_2$, a gas flow cell is interconnected with a

proportional counter tube (type LB 6280). An equivalent of 12 - 20 mmol of carbon as organic matter is mineralised in the anodic compartment at 135°C.

Results and Discussion

The procedure described here is an useful tool for the mineralization of hazardous, radioactively contaminated organic waste including C-14-labelled components. Only halogen compounds may interfere with this reaction. In this process the formation of additional hazardous waste is prevented by continuous electrolytic regeneration of chromium-III to chromium-VI. Fig. 1 presents the time-resolved C-14 carbon dioxide evolution during mineralization of some C-14-labelled organic compounds.

Fig. 1: Kinetics of anodic mineralization of the some carbon-14-labelled organic compounds



Acknowledgements

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References

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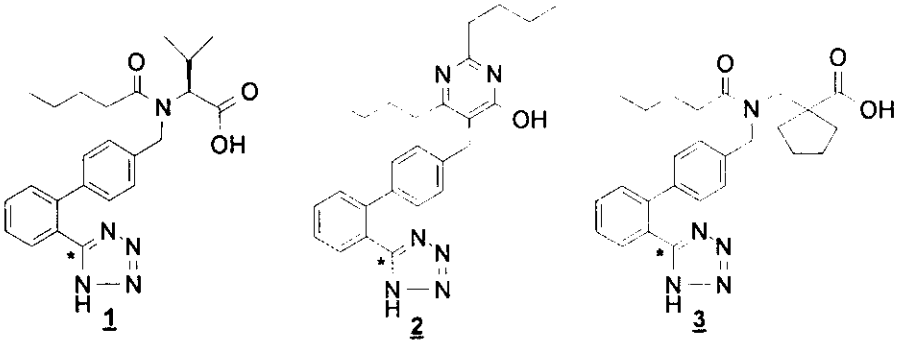
Synthesis of C-14 labelled DIOVAN ™ (part 1) [1]

P. Ackermann *, N. Wigger, P. Leupp, H. Mory
Novartis Crop Protection AG, RS-ILS, Basel, Switzerland

For pharmacokinetic and metabolic investigations **DIOVAN**™ **1** as well as the two related compounds **2** and **3** had to be labelled with C-14. **DIOVAN**™ belongs to a class of orally active specific angiotensin II antagonists [2] which has successfully been introduced into the market for the treatment of hypertension.

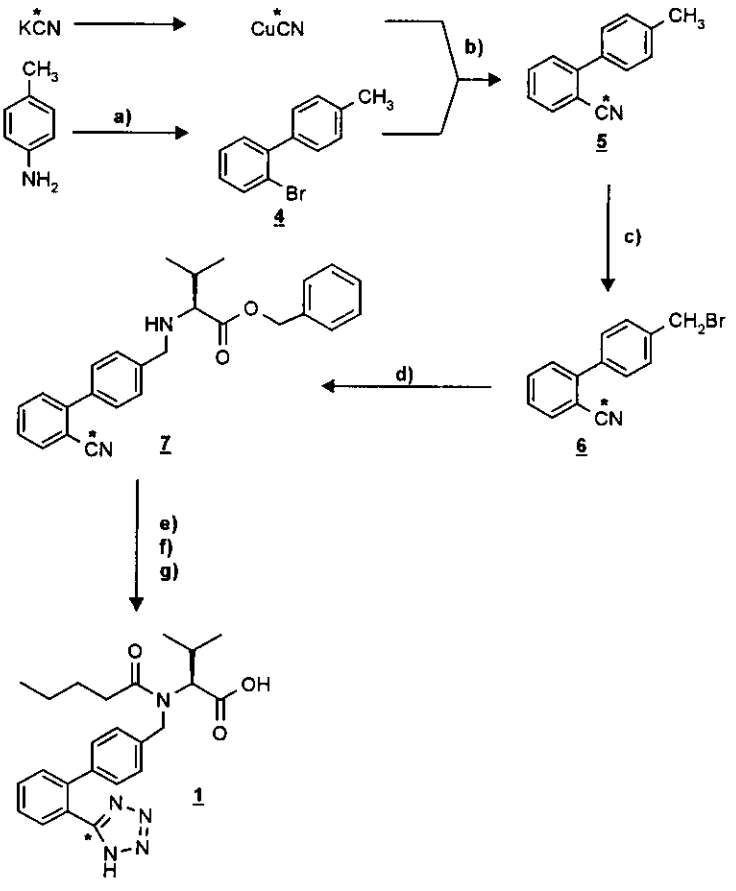


1 **DIOVAN™** **2** **3**

We used 4'-methyl-biphenyl-2-[^{14}C]carbonitrile **6** as a common intermediate for the preparation of the labelled molecules **1**, **2**, **3**.

The synthesis of **6** was accomplished in three steps starting from p-toluidine: diazotization, Gomberg-Bachmann coupling of the alkaline diazonium salt solution to bromobenzene to give 2-bromo-4'-methyl-biphenyl **4** [3] and replacement of the halogen upon treatment with Cu^{14}CN. Reaction of **6** with an appropriate amine or with a β-ketoester led in four steps to the desired molecules **1** to **3**.

[^{3}H]DIOVAN™ was prepared from an unsaturated precursor by catalytic hydrogenation using Pd-C in THF. The two enantiomers of **1** were easily resolved on a CHIRALCEL OD column using a mixture of n-hexane : 2-propanol : TFA = 85:15:0.1 as mobile phase.



Reaction conditions: **a)** $NaNO_2$, HCl, H_2O, 0°C, bromobenzene, [3]; **b)** Cu*CN, DMF, reflux, 32h, 85%; **c)** NBS, AIBN, CCl_4, reflux, 30min., 79%; **d)** valine benzyl ester, DMF, 80°C, DEA, 90min, 85%; **e)** valeryl chloride, DEA, toluene, 2h, 98%; **f)** Bu_3SnN_3, 24h,140°C, 95%; **g)** 10% Pd/C, H_2, dioxane, 50h, 65%.

References:

[1] Moenius Th . et.al. following communication

[2] Bühlmayer P. et.al. *Bioorganic and Medicinal Chemistry Letters* **4:** 29-34 (1994)
Waldmeier F. et.al. *Xenobiotica* **27:** 59-71 (1997)

[3] Gomberg M. et. al. *J. Am. Chem. Soc.*, **46**, 2339 (1924).

Preparation of Carbon-14 Labelled BAY x 3702 for Intravenous Administration in Man

Dietrich Seidel, Bayer AG, PH-PD P Drug Metabolism & Isotope Chemistry

For better understanding of the metabolism of BAY x 3702 a study with radioactively labelled substance in man was necessary.

Carbon-14 was selected as radioactive isotope.

Special requirements were given by
- the pharmacological properties of the drug,
- the Radiation Exposure Evaluation,
- the intravenous administration of the drug.

So a uniform labelling was excluded and the compound was needed with two different specific radioactivities.
Starting from commercially available [carbonyl-^{14}C]2-hydroxy-acetophenone (11.1 GBq) the condensation and subsequent ring closure [1] and the hydrogenation [2] were performed as described in the literature under optimized conditions. The separation of the enantiomers was carried out successfully by semipreparative HPLC on a chiral column with reworking of the undesired enantiomer by racemization. The following aminolysis with benzylamine gave no loss of enantiomeric excess. After by reduction with Red-Al® the last intermediate was produced by alkylation with bromobutyl-saccharin. The final product was formed by hydrogenolytical debenzylation and subsequent addition of hydrochloric acid. After repeated purification by semipreparative HPLC [4-chroman-^{14}C]BAY x 3702 was obtained with a radiochemical and chemical purity > 99 %. The reaction sequence with chemical details is shown in the following scheme. The desired product was obtained with a total radioactivity of 399 MBq which corresponds to 6.3 % of the theory with respect to the use of only 2/3 of the last intermediate.

All synthetic steps and all starting materials were well documented. Because of the administration route and the known hydrolysis (saccharinyl moiety) under alkaline or neutral conditions a solution of the labelled compound in 0.01 M hydrochloric acid was used as drug substance. Two batches were prepared with the same substance content of 1 mg/ml and different specific radioactivities of 2.58 MBq/mg and 1.89 MBq/mg and checked analytically according to an authorised certificate. The results were in good compliance with the requirements and the batches were released for preparation of the drug formulation.

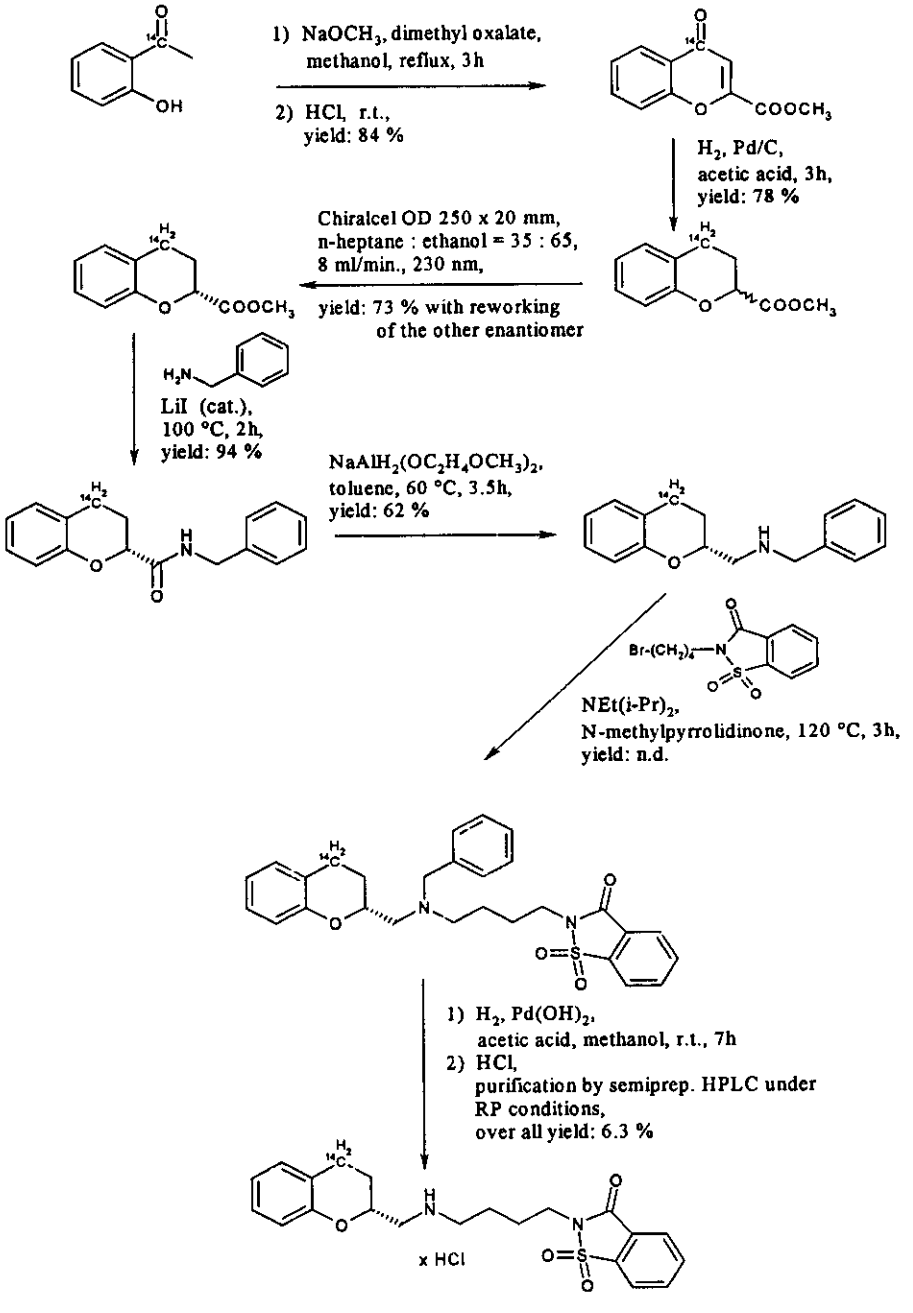
The preparation of the drug formulation was performed by filtration sterilisation of the solution under sterile room technology after process validation with non-labelled material. The results of a new analytical check under authorised conditions, including test of sterility, were in good compliance with the specification as well.

References

1) Ellis, G.P. and Thomas, I.L. – J. Chem. Soc., Perkin Trans. 1 (1974) 2570 (non-labelled)

2) Augstein, J., Monro, A.M., Potter, G.W.H. and Scholfield, P. – J. Med. Chem. 11 (1968) 844 (non-labelled)

Reaction scheme



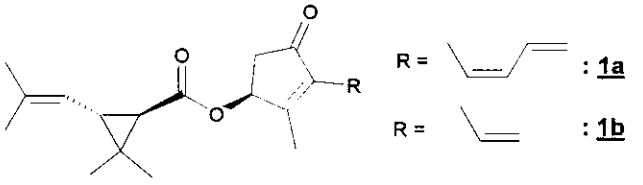
[4-chroman-^{14}C]BAY x 3702

Some Syntheses of C-14 Labelled Pyrethrins

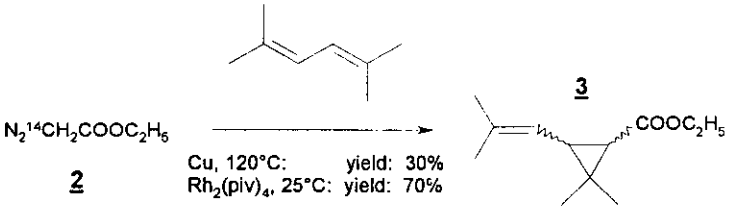
A. Jon Bloom and Peter M Winton

Custom Labelling and Special Synthesis, Amersham Pharmacia Biotech
Forest Farm, Cardiff, CF14 7YT, UK

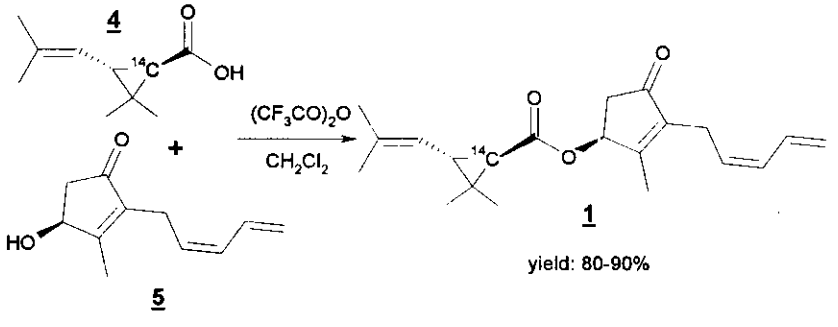
The standard route to the natural product insecticide [cyclopropane-1-^{14}C]pyrethrin I **1** suffers from low yields and multiple isomer formation.



We have re-investigated the synthesis and made several improvements to the chemistry. Our first improvement concerned the reaction of ethyl [2-^{14}C]diazoacetate **2** with 2,5-dimethyl-hexa-2,4-diene to give ethyl [cyclopropane-1-^{14}C]chrysanthemate **3**. Use of the organic soluble catalyst rhodium(II)pivalate [1] instead of the more usual copper powder gave an improved yield. The esters thus obtained were a 1:1 mixture of *cis* and *trans* isomers. The ratio was converted to 1:19 by the use of a $PdCl_2(C_6H_5CN)_2$ catalyst [2].



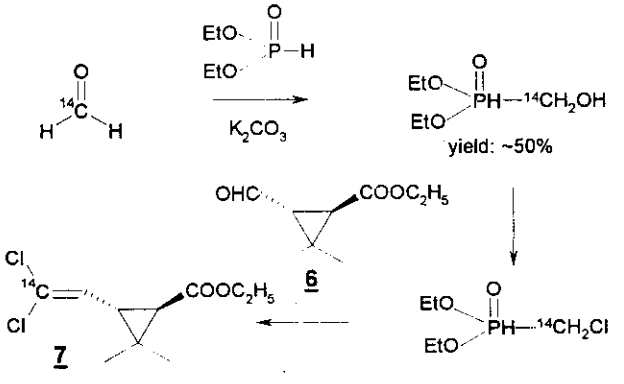
The final step to make pyrethrin I was the reaction of [cyclopropane-1-^{14}C]-chrysanthemic acid **4** with pyrethrolone **5**. A low yield was obtained upon the



conversion of the acid into the acid chloride followed by reaction with **5** and base. However, an excellent yield was obtained simply by mixing the acid with the alcohol component and adding trifluoroacetic anhydride

The analogue of pyrethrin I **1a**, allethrin **1b**, was prepared labelled in the alcohol (allethrolone) portion by the method of Schecter [3] . The compound could be made enantiomerically pure by **(i)** hplc resolution using a CHIREX 3019 column eluting with hexane-dichloroethane-ethanol (440:60:0.15), **(ii)** hplc resolution of the [^{14}C]allethrolone on Chiralpak AD eluting with hexane-ethanol (19:1) or by lipase resolution of [^{14}C]allethrolone acetate followed by dilute HNO_3 hydrolysis of the unreacted acetate. The configuration of the antipodal alcohol was inverted by mesylation followed by basic hydrolysis with $Ba(CO_3)_2$ [4].

[cyclopropane-1-^{14}C]Chrysanthemic acid **4** can be used to make halogeno-substituted analogues [5]. Oxidative cleavage of the double bond provides aldehyde **6** which reacts with suitably substituted Wittig reagents to give, for example, 'NRDC acid' **7**. Interestingly, when we made 'NRDC acid' labelled in a different position using a labelled version of the Wittig reagent of Savignac et al [6], we found a reduction in specific activity from 53 to 29 mCi/mmol.



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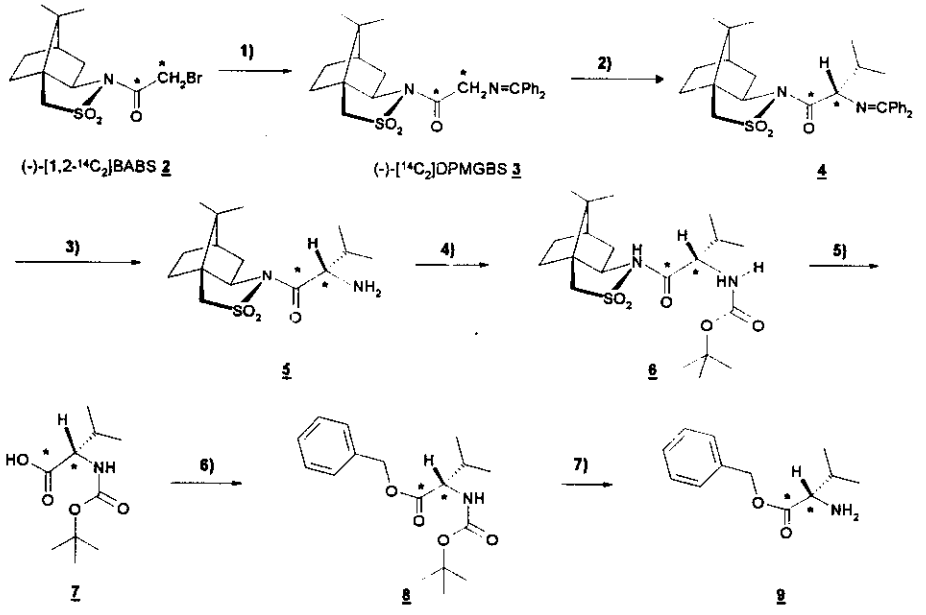
Synthesis of C-14 Labelled DIOVAN ™ (part 2) [1]

Th. Moenius *, R. Voges*, P. Burtscher, Ch. Zueger

NOVARTIS Pharma Ltd., Preclinical Safety, DMPK-Isotope Section, Basel, Switzerland

Doubly C-14 labelled **DIOVAN™** 1, a specific angiotensin II receptor antagonist, developed for a superior treatment of hypertension [2], was requested for drug-drug interaction studies. Labelling of 1 in the valine moiety required [1,2-^{14}C]valine benzyl ester 9 as a key intermediate, which was accessible in a diastereoselective synthesis starting from (-)-bromo[1,2-$^{14}C_2$]acetyl bornane-10,2-sultam 2 ([$^{14}C_2$]-BABS) [3] by alkylation of (-)-[$^{14}C_2$]DPMGBS 3 with isopropyl iodide, exchange of the N-protective group, cleavage of the auxiliary, and EDCI-mediated esterification with benzyl alcohol.

Scheme 1: Synthesis of L-[1,2-^{14}C]valine benzyl ester 9

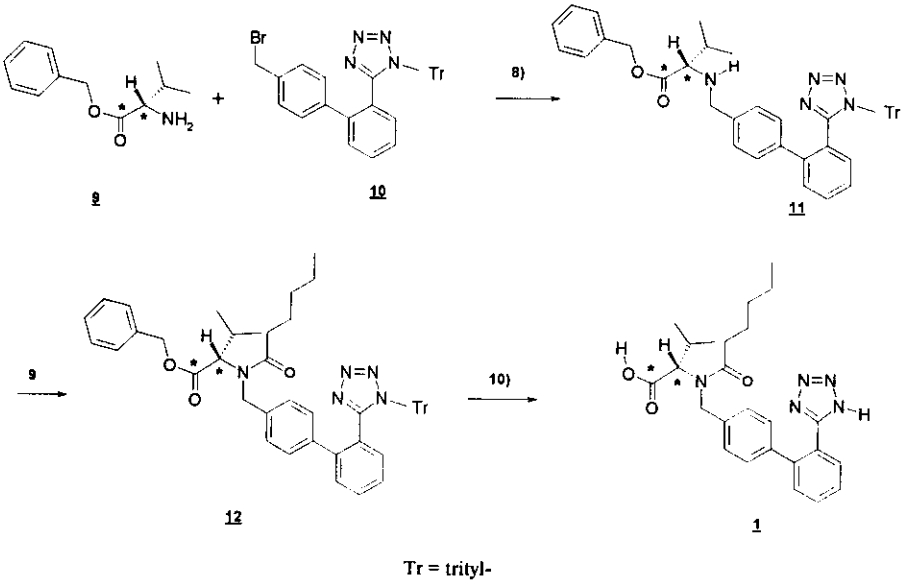


Reaction conditions: 1) Ph_2C=NH, CH_3CN, 70°C, 3.5 h, 75%; 2) n-BuLi, THF, DMPU, -78°C, 30 min., 2-iodopropane, -78°C/15 min, r.t./12 h, 76%; 3) 1N HCl - THF 1 : 1, r.t., 2 h, 85%; 4) $(BOC)_2O$, THF, r.t., 12 h, 99%; 5) 1N LiOH : THF 1 : 2, r.t., 2 h, 100%;6) BzOH, DMAP, EDCI, CH_2Cl_2, r.t., 90 min., 65%; 7) TFA, CH_2Cl_2, r.t., 60 min., 100%;

Mono-N-alkylation of 9 with bromide 10, N-acylation with valeryl chloride, and concluding deprotection resulted in [$^{14}C_2$]DIOVAN ^{TM} 1 in an overall radiochemical yield of 10%.

The optical purity of 1 was determined with > 98.5 %ee by chiral-HPLC.

Scheme 2: Synthesis of [$^{14}C_2$] DIOVAN ^{TM} 1



Reaction conditions: 8) DMF, *N,N*-diisopropylethylamine, 80°C, 150 min., 67%; 9) valeryl chloride, *N,N*-diisopropylethylamine, toluene, 81%; 10) Pd/C 10%, EtOH, H_2, 40°C, 240 min., recrystalization EtOAc : n-hexane, 60%;

In addition to the sucessful synthesis described above also failures will be discussed in the full paper [4].

References:

[1] Ackermann P. et.al. preceding communication

[2] Bühlmayer P, et.al. *Biorganic and Medicinal Chemistry Letters* **4**: 29-34 (1994), Waldmeier F. et.al. *Xenobiotica* **27**: 59-71 (1997)

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[4] Moenius Th., Ackermann P. full paper in preparation

Labelling of HTF919 with Stable and Radioactive Isotopes

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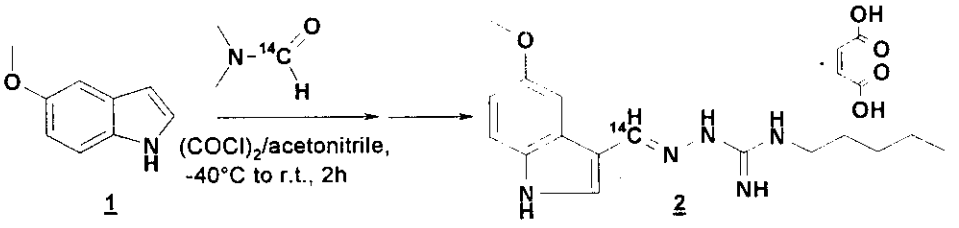
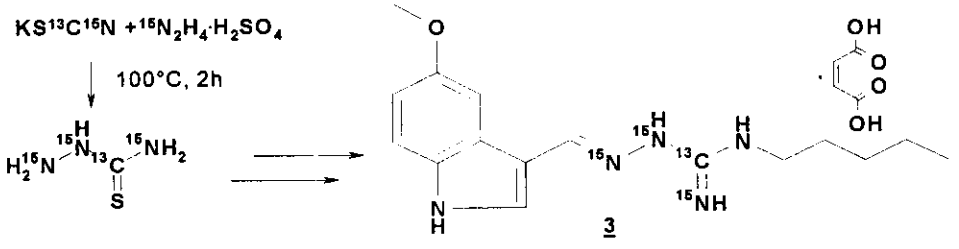
HTF919 [tegaserod, 5-methoxy-indole-3-carboxaldehyde amino(pentylamino)methylene hydrazone], a selective 5-HT_4 receptor partial agonist is currently being developed in the treatment of gastrointestinal motility disorders. The exploratory pharmacokinetic studies on **HTF919** (structure **2**, unlabelled) were performed with the carbon-14 label in the methylene-position of the side chain. The label was introduced in a Vilsmayer reaction using equimolar amounts of 5-methoxy-indole **1** and labelled dimethylformamide in acetonitrile. Labelled dimethylformamide was converted to the active Vilsmayer-reagent with oxalyl chloride. Several solvents (DMSO, sulfolane, N-methylpyrrolidone, dimethylacetamide, THF, methylene chloride, acetonitrile) were tested in the initial formylation reaction. Only acetonitrile gave a nearly quantitative yield of the corresponding indole-3-aldehyde.



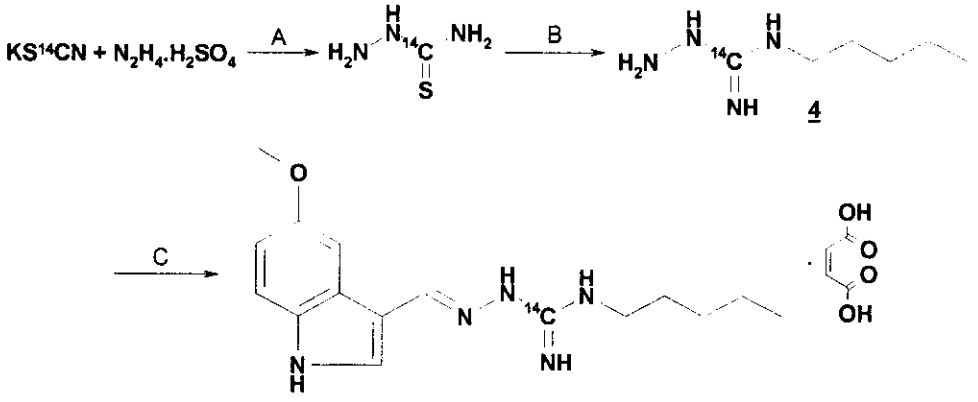
1 **2**

The analysis of clinical samples required a stable isotope labelled molecule **3** with M+4 mass units. Commercially available doubly labelled potassium [^{13}C,^{15}N]thiocyanate and [$^{15}N_2$]hydrazine sulfate were employed as starting materials for thiosemicarbazide (M+4) which was obtained in over 80 % yield after repetitive heating of the mother liquors containing the unreacted components.



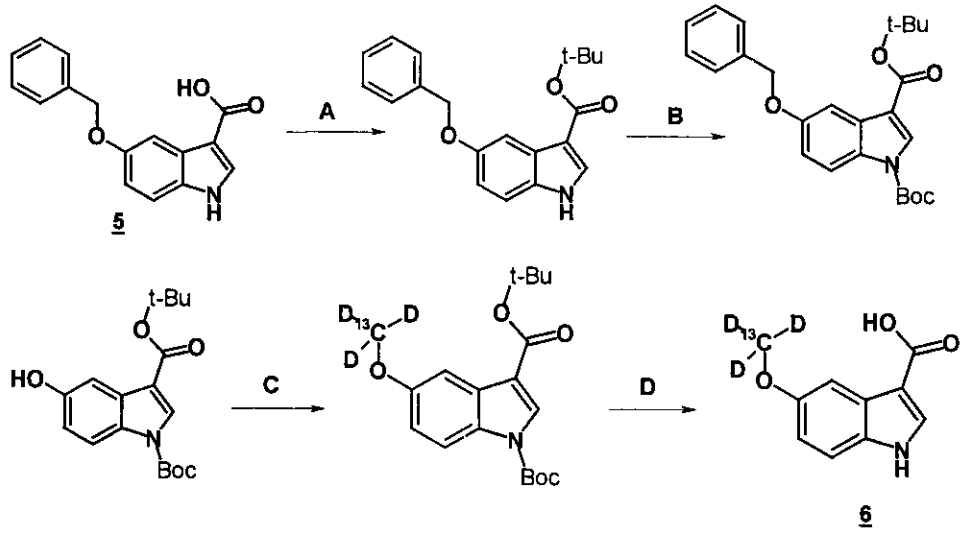
3

A major metabolic pathway was the cleavage of the carbazimidamide (= amino-guanidine) part which prompted us to study the fate of this moiety labelled in the carbazimide-carbon. The synthesis started from labelled thiosemicarbazide (from $KS^{14}CN$ and hydrazine sulfate). The intermediate N-pentylaminoguanidine **4** was isolated using ion exchange resin methodology followed by preparative reverse phase chromatography.



Conditions: **A**: 100°C, 2h; **B**: CH_3I, methanol, reflux, followed by n-pentylamine/reflux, 4h; **C**: 5-methoxy-indole-aldehyde, MeOH, pH 3.5, 2.5 h, r.t.; salt formation with maleic acid in acetone

An intermediary metabolite of tegaserod is 5-methoxy-indole-3-carboxylic acid (**5**, unlabelled) . For its determination in plasma and urine an internal standard with M+4 was needed as well. The first attempt to use 5-hydroxyindole-3-aldehyde as O-demethylated precursor failed since the remethylation gave O- and N-methylation. Furthermore, the envisaged oxidation of the aldehyde to the acid proved to be difficult and gave low yields. The alternative synthesis started from 5-benzyloxyindole-3-carboxylic acid **4** which was protected by esterification with dimethylformamide-t-butylacetal and by N-bocylation. Debenzylation with Pd/C and remethylation with $^{13}CD_3I$ followed by deprotection with trifluoroacetic acid gave finally the desired labelled metabolite in excellent yields.



Conditions: **A**: dimethylformamide di t-butylacetal, toluene, 100°C; **B**: $(Boc)_2O$, cat. DMAP, THF r.t.; **C**: H_2/Pd-C, ethyl acetate - 5% methanol, r.t. 4 h; **D**: $^{13}CD_3I$, acetone, K_2CO_3

SYNTHESIS OF A POTENTIAL TRACER FOR CANCER IMAGING : 16α-[^{18}F]FLUORO-ESTRADIOL-3,17β-DISULPHAMATE ([^{18}F]FESDS)

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[2] Hans-Knöll-Institute of Natural Product Research, D-07745 Jena, Germany

SUMMARY

The synthesis of [^{18}F]FESDS as a potential tracer for positron emission tomographic investigations is described. 16α-[^{18}F]Fluoroestradiol ([^{18}F]FES) is converted with excessive sulphamoyl chloride in absolute acetonitrile in presence of an alkali. Using kryptofix 2.2.2 and K_2CO_3 as alkali, [^{18}F]FESDS was obtained in yields of 50 – 60%.

Keywords: Synthesis, steroids, ^{18}F-labelling, PET-tracer

INTRODUCTION

Sulphatase inhibitors prevent the formation of free estrogen in tissues as breast cancer in which high sulphatase activity occur. 16α-Fluoroestradiol-3,17β-disulphamate (FESDS) is a high-effective steroid sulphatase inhibitor and could possibly serve as chemotherapeutics against breast cancer. On the other hand, [^{18}F]FESDS was thought to be able to image sites of high sulphatase activity in PET investigations. Therefore, it was necessary to develop the synthesis of [^{18}F]FESDS of high specific activity. The reaction is shown in FIGURE 1 .

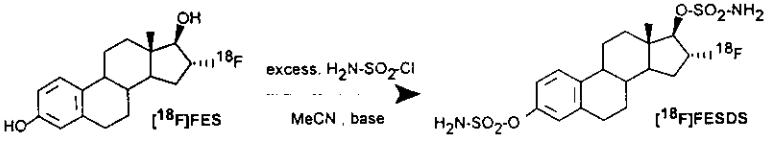

FIGURE 1

RESULTS and DISCUSSION

When reacting with 16α-fluoroestradiol (FES), sulphamoyl chloride (H_2N-SO_2-Cl, SCl) forms three different sulphamates. These compounds were first synthesized and characterized in our department (1). To obtain preferably 16α-fluoroestradiol-3,17β-disulphamate (FESDS), excessive SCl had to be applied. FES was dissolved in anhydrous MeCN and after adding Na_2CO_3 and SCl, the heterogeneous reaction mixture was vigorously stirred at room temperature.

In the radioactive experiments, these conditions produced only polar by-products. Therefore, 2,6-di-tert.butyl-4-methylpyridine (DBMP) was applied as alkali. Complete conversion of [^{18}F]FES was observed on evaporating the reaction mixture at 70 °C. However, mainly polar products (75 %) and only 25 % [^{18}F]FESDS were found.

Further investigation showed that kryptofix 2.2.2 together with the equivalent amount of K_2CO_3 is a very useful alkali. The experiments showed a significant decrease of the amount of polar by-products. The yield of [^{18}F]FESDS was between 50 and 60 % (FIGURE 2). Further experiments confirmed that the procedure is suited for reproducible preparation of [^{18}F]FESDS. RP-18 column purification gave a product with a radiochemical purity of > 99 %.

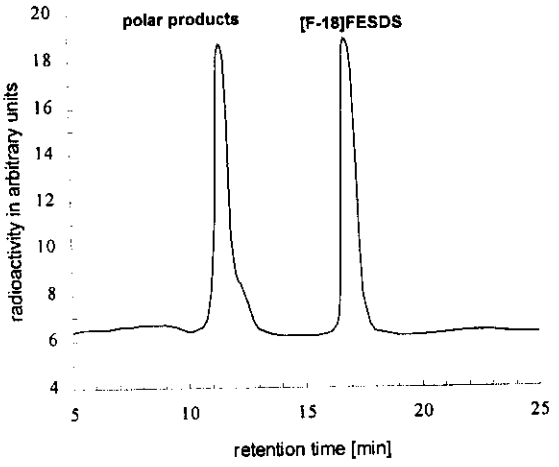

FIGURE 2 : Preparation of pure [F-18]FESDS on a preparative RP-18 column after sulphamoylation in presence of kryptofix 2.2.2/potassium carbonate

EXPERIMENTAL

About 100 – 500 MBq of no-carrier-added [^{18}F]FES (2) dissolved in about 2 ml 55% EtOH was placed in a bulb. After adding a solution of 22,5 mg kryptofix 2.2.2 and 4,2 mg K_2CO_3 in 1,5 ml MeCN, the solvent was removed with the aid of absolute MeCN on a rotary evaporator. A solution of 10 mg SCl in 2 ml MeCN was added to the absolutely dry reaction batch. The bulb was stirred at 70 °C. Samples of 10 µl were investigated in an analytic HPLC system. To work up the reaction batch, the solvent was removed and a 0,5 M solution of ammonium acetate in 55% EtOH (0,6 ml) was added. The clear solution (0,5 ml) was injected into a preparative HPLC system. The column was eluted with 55% EtOH (flow: 1,5 ml/min). After u.v. detection at λ = 275 nm , the eluent was measured in a radioactivity detector The fraction of each peak was collected and measured in an ionisation chamber.

CONCLUSION

The sulphamoylation of [^{18}F]FES in the presence of kryptofix 2.2.2/K_2CO_3 is a simple, rapid, and reproducible procedure. The time expenditure for synthesis and column purification is about 30 min. The procedure is sufficient to be transferred into a remote-controlled and automated module without any problems. Only a module-assisted synthesis can guarantee high radioactivity amounts of [^{18}F]FESDS.

ACKNOWLEDGEMENT

This work is supported by Deutsche Forschungsgemeinschaft. We also thank Mr. Dr. U. Pleiss (BAYER AG) for his helpful discussions.

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DEVELOPMENT OF POTENTIAL TUMOUR IMAGING AGENTS BY 4-[^{18}F]FLUOROBENZOYLATION OF NEUROTENSIN ANALOGUES

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Key Words: Neurotensin(8-13), ^{18}F-labelling, [^{18}F]SFB, neurotensin receptor, pseudopeptide

SUMMARY

The 4-[^{18}F]fluorobenzoyl compounds of Neurotensin(8-13) (NT(8-13)) as well as [Arg^{8}Ψ(CH$_2$NH)Arg^9]NT(8-13) were obtained by reaction of N-succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB) with these peptides in aqueous buffered solutions at pH 8.3 in r.c.y. of up to 43 % (related to [^{18}F]SFB; decay-corrected) within 80 min (including HPLC purification). This is the first example for the specific radiolabelling of the α-amino group at the N-terminal arginine unit of peptides using [^{18}F]SFB. Receptor affinity and biodistribution studies of the labelled NT derivatives are currently underway.

INTRODUCTION

A promising approach for tracking tumours is the utilization of radiolabelled peptides with specific affinity to tumour associated receptors. Since neurotensin receptors were found in several tumour cell lines like small cell lung cancer [1], human colon cancer [2] and pancreas cancer [3], the development of ^{18}F-radiolabelled derivatives of neurotensin (NT = Pyr-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu) which bind with high affinity to these receptors has attracted our interest. Investigations on structure affinity relationship have shown that the C-terminal hexapeptide NT(8-13) is the shortest analogue of the parent peptide, displaying the full binding and pharmacological activities [4]. In order to develop a potential radiotracer for imaging of neurotensin receptors with positron emission tomography (PET) the hexapeptide NT(8-13) and its stabilized pseudopeptide [Arg^{8}Ψ(CH$_2$NH)Arg^9]NT(8-13) were used as starting materials [5].

A further purpose of this study was the evaluation of the suitability of [^{18}F]SFB for the first specific radiolabelling of non-lysine containing oligopeptides. Up to now the activated ester [^{18}F]SFB has only been used for labelling of peptides containing lysine in their sequences [6, 7, 8].

RESULTS AND DISCUSSION

The synthesis of [^{18}F]SFB started from the triflate salt of ethyl 4-trimethylammonium benzoate *via* a three-step procedure [9]. The kryptate supported nucleophilic substitution of [^{18}F]F$^-$ for the Me$_3$N$^+$ group is followed by saponification of the ethyl ester. In the final step O-(N-succinimidyl) N,N,N',N'-tetramethyluronium tetrafluoroborate was used as activating agent to give [^{18}F]SFB in r.c.y. up to 48 % (decay-corrected) related to [^{18}F]F$^-$ within 100 min.

Our labelling experiments revealed that [^{18}F]SFB reacts with the α-amino group of the N-terminal Arg sequence of peptides like the small model peptide Arg-Tyr, the NT(8-13) (according to Fig. 1) or the pseudopeptide [Arg^{8}Ψ(CH$_2$NH)Arg^9]NT(8-13) with reasonable to good chemoselectivities in aqueous buffered solutions at pH values between 7.2 and 8.3. Higher pH values gave rise to a mixture of more different radioactive products. For a complete consumption of [^{18}F]SFB we found it advisable to heat the mixture at 35 – 40 °C for 20 min. The labelled peptides were purified by semipreparative reversed-phase HPLC.

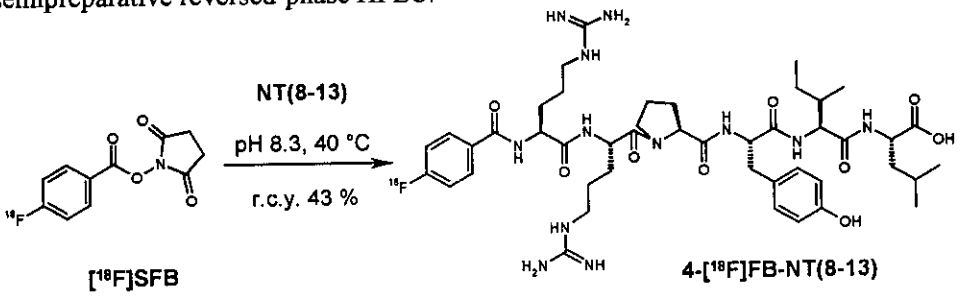

Figure 1: ^{18}F-Labelling of NT(8-13) with [^{18}F]SFB

EXPERIMENTAL

A solution of NT(8-13) or [Arg^{8}Ψ(CH$_2$NH)Arg^9]NT(8-13) (0.6 mg; 0.7 μmol) in 100 μl borate buffer (pH 8.3) was added to a solution of [^{18}F]SFB prepared according to [9] in 10 μl MeCN. The mixture was heated at 40 °C for 20 min. After dilution with 900 μl eluent the reaction mixture was subjected to chromatographic purification using semipreparative reversed-phase HPLC (Merck, LiChrospher WP300, RP 18, 12 μm; 250 mm × 10 mm; 24.9 % MeCN / 74.9 % water / 0.2 % TFA). The radioactivity corresponding to the desired product was collected and diluted with water. This solution was passed through an RP-18 cartridge. After washing with water, the purified ^{18}F-labelled peptide was eluted from the cartridge with EtOH.

The ^{18}F labelled peptides were identified by chromatographic comparison with reference compounds. The reactions carried out with nonradioactive SFB were shown to give the corresponding 4-FB peptides of which their structural identity was confirmed by proton NMR and MS studies.

ACKNOWLEDGEMENTS

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OXYGEN - 17 LABELLING EXPERIMENTS FOR INVESTIGATIONS ON MOLECULAR REARRANGEMENTS

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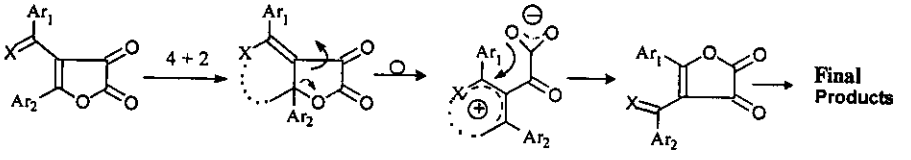
Summary

Unusual molecular rearrangements resulting from reaction of 4,5-aryl substituted furan-2,3-diones as well as thiophene-2,3-diones with several dienophiles are made evident by ^{17}O-labelling experiments through comparing the location and distribution of the label within educts and final products employing ^{17}O-NMR measurements. The labelling of the starting materials is easily achieved by simple exchange processes utilizing ^{17}O-labelled water.

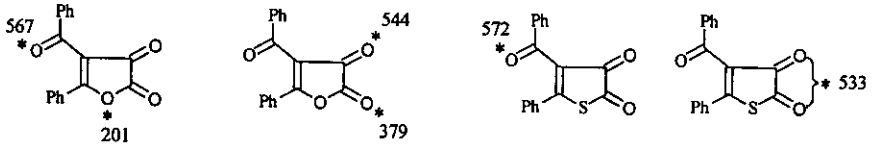
Introduction

The oxa(thia)-1,3-diene moieties of suitable aryl substituted furan(thiophene)-2,3-diones add several dienophiles (C-heterocumulenes, S-heterocumulenes, *Schiff* bases) via formal 4+2 cycloaddition reactions. The primary adducts in most cases are not stable and undergo unexpected rearrangements which could first of all be detected and verified by structural elucidation of the corresponding stable final products with aid of several single-crystal X-ray analyses[1]. Reasonable reaction pathways should run via intermediates in which the two oxygens of the former lactone group should get equivalent. In order to confirm this mechanistic proposal ^{17}O-labelling experiments controlled by ^{17}O-NMR measurements should be helpful in comparing the number of signals and chemical shift values of specifically labelled educts and final products.



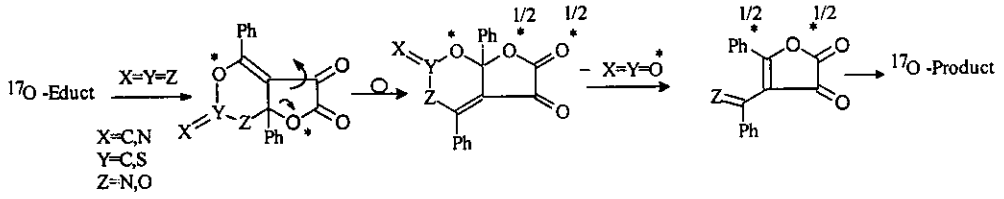
Results and Discussion

In order to ensure the assignments of chemical shift values for all oxygens found in educts and rearranged products two differently labelled educts have been prepared via simple exchange processes applying [^{17}O]water. Thus carbonyl groups of the starting materials (dibenzoylmethane or oxalic acid) were labelled utilizing the well-known *Erlenmeyer* hydration equilibrium [2], the ^{17}O-labelled thiophenediones were obtained from reacting the corresponding furandiones with H_2S.

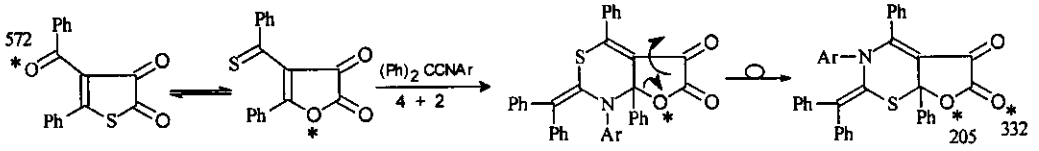


Numbers in *ppm*

C-Heterocumulenes (isocyanates, carbodiimides, ketene imines)[3], N-sulfinylamines [4] as well as N-alkylimines[5] in reaction with labelled furane-2,3-diones exactly follow the proposed mechanistic pathway including an unexpected novel furandione- rearrangement :



Similar surprising transformations, including an unusual long-range *Dimroth*-rearrangement, were observed by reaction of 4-benzoyl-5-phenylthiophene-2,3-dione with N-aryl-diphenylketene imines to afford furo[3,2-e]thiazines [1c] :



Experimental

The ^{17}O NMR spectra were recorded on a Bruker AM 360 spectrometer equipped with 10 mm broad-band probes operating at 48.78 MHz. The instrument settings were 20-40 kHz spectral width, 4,6 or 8 K data points, 100 ms preaquisition delay and 100-200ms acquisition time. Chemical shifts are reported relative to external water at 22°C. The error is estimated to be ± 5 ppm due to signal broadening problems, probably caused by association and/or diffusion phenomena. Generally, 10^2-10^5 scans were accumulated, the labeling degree was 5 – 10 %.

Conclusion

^{17}O-Labeling has been found a valuable tool to elucidate molecular rearrangements when oxygen atoms change their positions in the course of the reaction. In particular, a novel furandione rearrangement became evident which can be regarded as a peculiar variation of the well-known nucleophilic substitution at a vinylic carbon [6]. In this particular case the leaving group (O-C=O) does not in fact leave the molecule but remains bounded and again closes the ring by an intramolecular nucleophilic attack.

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SYNTHESIS AND BIOSYNTHETIC INCORPORATION OF LABELLED PRECURSORS INTO THE PHENYLPHENALENONES

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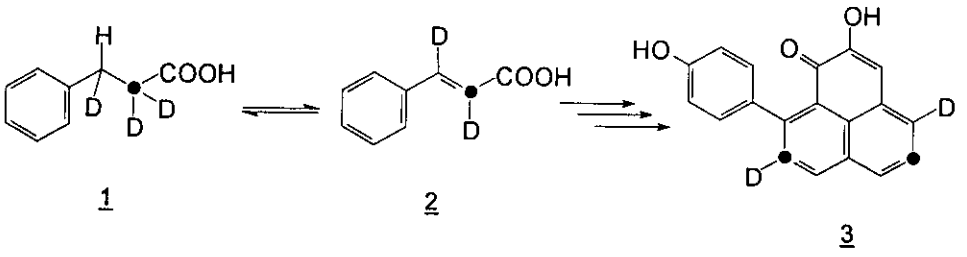
Summary

The synthesis of multiply labelled dihydrophenylpropanoids and phenylpropanoids and their utilisation in experiments on the reversible interconversion of those compounds and their biosynthetic incorporation into the phenylphenalenones, a group of unusual plant pigments is described.



1 2 3

Fig. 1 Reversible interconversion between phenylpropanoids and dihydrophenylpropanoids and biosynthetic incorporation of labelled precursors into phenylphenalenones in *Anigozanthos preissii*.

Introduction

Phenylphenalenones, a class of phenylpropanoid-derived natural products (1), are formed by *Anigozanthos preissii*, an Australian member of the Haemodoraceae plant family. Previous experiments indicated close relationship between the biosynthesis of phenylphenalenones and diarylheptanoids (2, 3). However, details of the biosynthetic pathway are still hypothetical. This paper describes the synthesis of labelled precursors and feeding experiments employing *in vitro* cultures of *A. preissii* and NMR spectroscopic analysis including direct HPLC-NMR coupling (4) to study the biosynthesis of the phenylphenalenones.

Results and Discussion

A number of ^{13}C- and multiply $^{2}H/^{13}C$-labelled precursors of the phenylpropanoid type were synthesised. The Erlenmeyer reaction was used to prepare [2-^{13}C]- and [2-^{13}C,2-^{2}H]cinnamic acid, singly labelled with ^{13}C, or doubly with ^{13}C and ^{2}H in position 2. Reduction of the cinnamoyl double bond by means of deuterium gas yielded dihydrophenylpropanoids, e.g. [2-^{13}C,2,2,3-$^{2}H_3$]dihydrocinnamic acid (1), containing a

multiply labelled saturated side chain moiety. Administration of that precursor to root cultures of *Anigozanthos preissii* followed by HPLC-^{1}H NMR analysis of crude extracts revealed the formation of various labelled isotopomers of cinnamic acid, e.g. (2), indicating reversible interconversion between compounds of the dihydrocinnamic acid and cinnamic acid pools. Moreover, NMR measurements of phenylphenalenones, e.g. hydroxyanigorufone (3), isolated from root cultures in the same feeding experiment, indicated retainment of ^{2}H in certain positions of the molecule and loss of ^{2}H in other positions. Detailed isotopomer analysis allowed conclusions on the mechanism of the phenylphenalenone biosynthesis (5).

Experimental

The biosynthetic experiments described here were carried out using root cultures of *Anigozanthos preissii*. [1-^{13}C]-and [2-^{13}C]cinnamic acid were synthesised from [1,3-^{13}C]- and [2-^{13}C]malonic acid, respectively, by the Erlenmeyer reaction using standard procedures. [2-^{13}C]Dihydrocinnamic acid was prepared from [2-^{13}C]cinnamic acid by hydrogenation at room temperature using 10% Pd-C as a catalyst in dry MeOH. [2-^{13}C,U-^{2}H]Malonic acid was prepared from [2-^{13}C]malonic acid by H/D exchange, periodically adding and evaporating D_2O (four times). Labelled malonic acid prepared by this procedure was subjected to the Erlenmeyer synthesis to yield [2-^{13}C,2-^{2}H]cinnamic acid. Both [2-^{13}C,2-^{2}H]- and [2-^{13}C]cinnamic acid were deuterated by D_2 gas under the conditions described above except the utilisation of MeOD as solvent. By this manner [2-^{13}C,2,3-$^{2}H_2$]- and [2-^{13}C,2,2,3-$^{2}H_3$]dihydrocinnamic acid (1) were obtained in quantitative yield. NMR spectra were measured on a Bruker DRX 500 NMR Spectrometer at 500.13 MHz (^{1}H) and 125.75 MHz (^{13}C). HPLC-NMR experiments were carried out in the stopped flow mode using a 4 mm inverse detection probehead.

Conclusions

Feeding experiments have shown for the first time reversible interconversion between phenylpropanoids and dihydrophenylpropanoids in plants. Details of the biosynthesis of the phenylphenalenones have also been elucidated.

Acknowledgements

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Application of $^{14}CO_2$ Absorption on Molecular Sieve to a new Manifold System

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Concept

CO_2 can be reversibly adsorbed on molecular sieve at room temperature. Heating of the trap containing molecular sieve with adsorbed CO_2 results in an equilibrium pressure depending on the temperature. The chemical reaction with $^{14}CO_2$ is performed in a Manifold System of stainless steel. After the reaction surplus $^{14}CO_2$ can be reabsorbed on the trap for further use.

Storage of $^{14}CO_2$

Traps for $^{14}CO_2$ storage are made from stainless steel and filled with commercially available molecular sieve.The molecular sieve is activated by heating to 450 °C at high vacuum for 2 hours.

Experiments with CO_2 showed, that 1.6 mmol CO_2 per gram molecular sieve are nearby completely adsorbed at room temperature. At 400 °C more than 90 % of the adsorbed CO_2 is expanded into a connected volume of 51 ml. Diagram 1 shows the equilibrium pressure of 3.2 mmol CO_2 adsorbed on 4.4 grams of molecular sieve in a volume of 51 ml depending of the temperature of the molecular sieve.

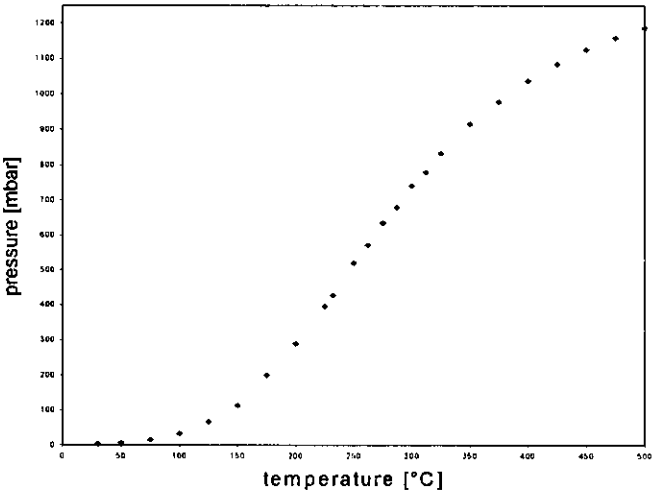

Diagram 1: Equilibrium pressure of 3.2 mmol CO_2 on 4.4 gram molecular sieve in 51 ml.

$^{14}CO_2$ Manifold System

We designed a high vacuum Manifold System for the handling of $^{14}CO_2$, based on our long experience with tritium handling systems. Diagram 2 shows a Manifold System with connections to a vessel for the production of $^{14}CO_2$, two traps with molecular sieve and to the reaction branch with a distillation ampoule or a NaOH trap. Furthermore there is supply of an inert gas, a pirani and an absolute pressure sensor 0 – 4000 mbar.

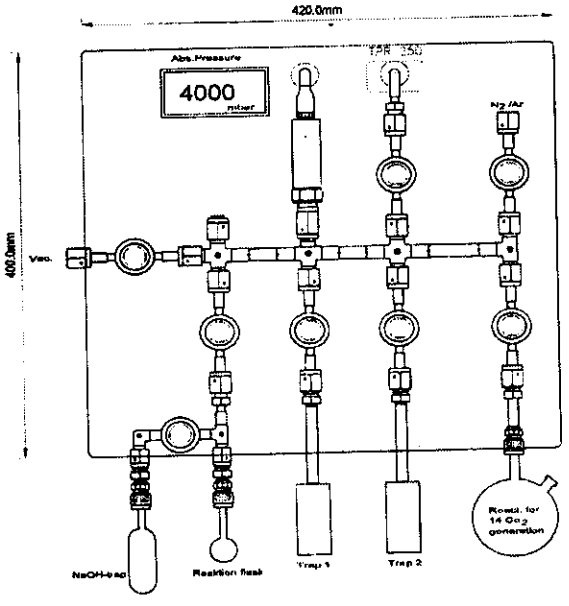

Diagram 2: $^{14}CO_2$ Manifold System

The reaction branch with two connections offers the option to freeze the solvents of the reaction into a separate ampoule. It also provides the possibility to precipitate surplus $^{14}CO_2$ in a solution of NaOH if the melting point of the solvent prevents to take the gas back on the molecular sieve trap. The molecular sieve traps can be made in various diameters up to 28 mm and a height of 70 mm. They can be loaded with up to 25 grams of molecular sieves, so a maximum quantity of 40 mmol $^{14}CO_2$ can be stored in each trap. The use of two traps on the Manifold System offers additional safety and manipulation options.

Advantages of the Stainless Steel Manifold System

- $^{14}CO_2$ must not be generated for each experiment.
- The $^{14}CO_2$ pressure can be adjusted by the temperature of the molecular sieve.
- Option to work with $^{14}CO_2$ overpressure.
- Surplus $^{14}CO_2$ can be recovered from the vacuum system after the reaction. Discharges to the environment are reduced to a minimum.
- All stainless steel components guarantee highest safety and reliability at maximum leaktightness.

Synthesis of complex radiolabeled carbohydrates by use of biological systems: Two examples

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Summary

The enzymatic activity of different organisms can be used to synthesize complex radiolabeled compounds from cheap and available substrates. The synthesizing steps can either be performed *in-vivo* by using genetically optimized microorganisms or *in-vitro* by using purified enzymes. Both strategies were successfully applied to synthesize different radiolabeled carbohydrates not available or difficult to synthesize by common methods.

In an example for the first strategy, we used a modified *Escherichia coli* strain to produce the labeled disaccharide trehalose [α-D-glucopyranosyl-1->1-α-D-glucopyranoside] from glucose (1). The strain is able to transport glucose but neither to metabolize glucose nor the synthesized trehalose. Therefore, the transformation from glucose to trehalose is almost complete. After harvesting and lysis of cells, the product of the enzymatic reaction could be easily purified by chromatographic techniques. The overall yield of stereochemically and radiochemically pure trehalose from glucose was about 80%.

In an example for the second strategy, extracts from genetically modified *E. coli* cells were employed to synthesize *in-vitro* linear maltodextrins. Products of high specific activity up to maltooctaose were synthesized from maltose (2). By combining this first reaction with a second enzymatic cycling reaction, its also possible to synthesize cyclodextrins.

Knowing the variety of enzymatic activities in organisms and using the power of modern methods in molecular biology, highly efficient synthesis reactions of a broad spectrum of carbohydrates could be developed successfully, *in-vivo* or *in-vitro*.

Example I: [^{14}C]Trehalose

The synthesis of trehalose in a wild type *E. coli* strain occurs under osmotic stress and is subject to a complex enzymatic network [Fig. 1]. We therefore cloned the genes coding for the proteins involved in trehalose synthesis [OtsA and OtsB] in a plasmid under control of an inducible promoter [pRHo700]. This allows us to induce trehalose synthesis under different growth conditions. Enzymatic degradation of the synthesized trehalose was prevented by mutating two genes coding for a periplasmic and a cytoplasmic trehalase [*treA* and *treF*]. In *E. coli*, glucose enters the cell via a phosphotransferase system [PTS] as glucose-6-phosphate. The genetically engineered bacteria were unable to metabolize glucose-6-phosphate due to a block at the level of phosphoglucoisomerase [Pgi, glucose-6-P -> fructose-6-P]. Therefore, after addition of glucose to properly induced bacterial cells, almost all of the transported monosaccharide is used to synthesize trehalose. After harvesting and extracting the cells with 70% hot ethanol, the stereochemically uniform α,α-trehalose synthesized could be purified chromatographically [by paper chromatography]. Quantitative analysis was carried out with 350 μCi [U-^{14}C]glucose as starting material. It was calculated that less than 10% of the original radioactivity remained in the media and 10% in the cell pellet, yielding 80% of radioactive material as trehalose. Upscaling of this method (100-fold, 30 mCi) gave a total yield of about 50%.

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Example II: Linear and cyclic [^{14}C]maltodextrins ([^{14}C]cyclodextrins)

E. coli amylomaltase [MalQ] is an enzyme catalyzing the formation of long chain maltodextrins by a disproportionation following the scheme [Fig. 2]:

$$Glc_n + Glc_m <-> Glc_{n-x} + Glc_{m+x} \quad [n > 2;\ m, x \geq 1]$$

For the production of [^{14}C]dextrins with high specific activity, a dialyzed extract of a genetically modified *E. coli* strain overproducing Amylomaltase was incubated with [U-^{14}C]maltose. This strain also carries lesions in genes [*malP, malS, malZ*] whose products are involved in the degradation of maltodextrins. As analysed by TLC, the products of amylomaltase are linear maltodextrins at least up to maltooctaose.

To obtain [^{14}C]cyclodextrins, the reaction mixture or purified maltodextrins [$n \geq 8$] can be incubated with cyclodextrin glucosyltransferase from *Klebsiella oxytoca*. The kinetically favored product of this cyclization reaction is α-[^{14}C]cyclodextrin [cG6], but with cyclodextrin glycosyltransferases showing an appropriate specificity, also the synthesis of β- and γ-[^{14}C]cyclodextrins should be possible.

Conclusion

The synthesis of complex, radiolabeled carbohydrates by the use of biological systems is an viable alternative to chemical synthesis. By knowledge of the different metabolic and catabolic pathways in microorganisms, genetically optimized bacteria can be used as cellular factories to synthesize these compounds [*in-vivo* synthesis].

As a second strategy, the *in-vitro* synthesis of labeled carbohydrates by the use of purified enzymes combines several advantages of the *in-vivo* synthesis [e.g. the stereoselectivity of enzymes] with the simplicity to control a complex system of reactions.

These advantages can be used to synthesize different labeled carbohydrates with yet unknown qualities, as shown here for the case of stereochemical pure trehalose and linear or cyclic dextrins with very high specific activity.

Acknowledgments

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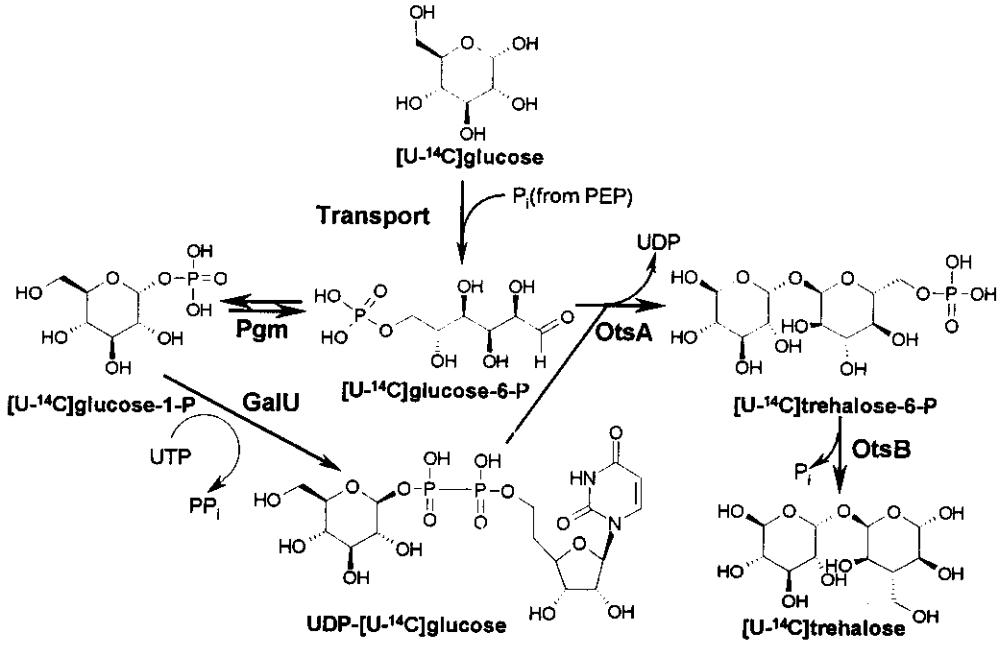

Fig.1: Synthesis of [^{14}C]trehalose *(E.coli)*

Pgm: phosphoglucomutase; **GalU:** UDP-glucose pyrophoshorylase; **OtsA:** trehalose-6-phosphate synthase; **OtsB:** trehalose-6-phosphate phosphatase

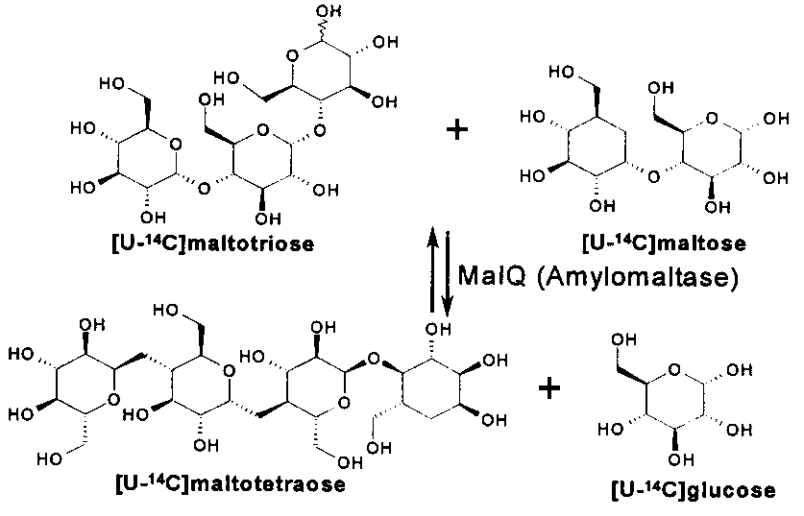

Fig.2: Synthesis of [^{14}C]maltodextrins and α-[^{14}C]cyclodextrin

principle of the disproportionation performed by amylomaltase [MAlQ] from *E. coli*