THE SYNTHESIS OF [U-^{14}C PHENYL] LS 840606, AN AGRICULTURAL FUNGICIDE.

G. Madegard, P. Mestre, P. Raimond[1], J-P. Noël*
CEA/Saclay, Service des Molécules Marquées, Département de Biologie Cellulaire et moléculaire
91191 GIF sur Yvette Cedex, FRANCE
[1] LETI (CEA - Technologies Avancées), DEIN/SPE/GCO 91191 Gif sur Yvette Cedex, FRANCE

SUMMARY

2,2',4'-Trichloro-[ring U-^{14}C]acetophenone **3** was the key intermediate of this synthesis patterned after the industrial route. An unexpected poor yield was observed during the preparation of **3** by the Friedel-Crafts reaction of chloroacetyl chloride with 1,3-dichloro-[U-^{14}C]benzene **10**, possibly the result of an isotope effect although this poor yield might be explained by other factors.

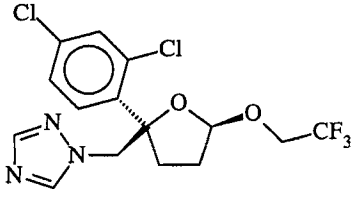
Two routes were checked for the preparation of 1,3-dichloro-[U-^{14}C]benzene **10**. The action of CCl_4 with 1,3-dinitro-[U-^{14}C]benzene at 280°C was entailed with explosions. A safer route started from [U-^{14}C]aniline via 2,4-dichloro-[ring U-^{14}C]acetanilide.

Friedel-Crafts reaction of **10** with acetyl chloride gave rise in 52% yield to 2',4'-dichloro-[ring U-^{14}C]acetophenone **16** which was brominated to 2-bromo-2',4'-dichloro-[ring U-^{14}C]acetophenone **17**; **17** was condensed with 2,2-(ethylenedioxy)ethylmagnesium bromide to compound **18**; **18** was condensed with 1,2,4-triazole to **5** then successively treated with HCl:water:dioxane and 2,2,2-trifluoroethanol/HCl. Separation of the two diastereomers by medium pressure liquid chromatography. 7% overall radioactive yield from [U-^{14}C]aniline. Radiochemical purity 99%.

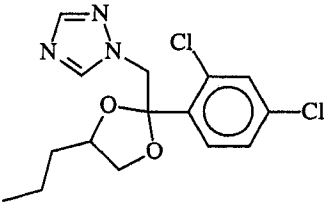
Key words: isotope effects, [ring U-^{14}C] labelling, 1,3-dichloro-[U-^{14}C]benzene, 2',4'-dichloro-[ring-^{14}C]acetophenone.

INTRODUCTION

LS 840606 **1**, (2*RS*,5*RS*)-5-(2,4-dichlorophenyl)-tetrahydro-5-(1*H*-1,2,4-triazol-1-ylmethyl)-2-furyl 2,2,2-trifluoroethyl ether, is a triazole fungicide discovered by Rhône-Poulenc Agrochimie. It inhibits the biosynthesis of sterols and is active against a wide range of pathogenic fungi [1]. Labelling with carbon-14 in the phenyl ring was required for metabolic and environmental studies.



(±)-1 : LS 840606



2 : Propiconazole

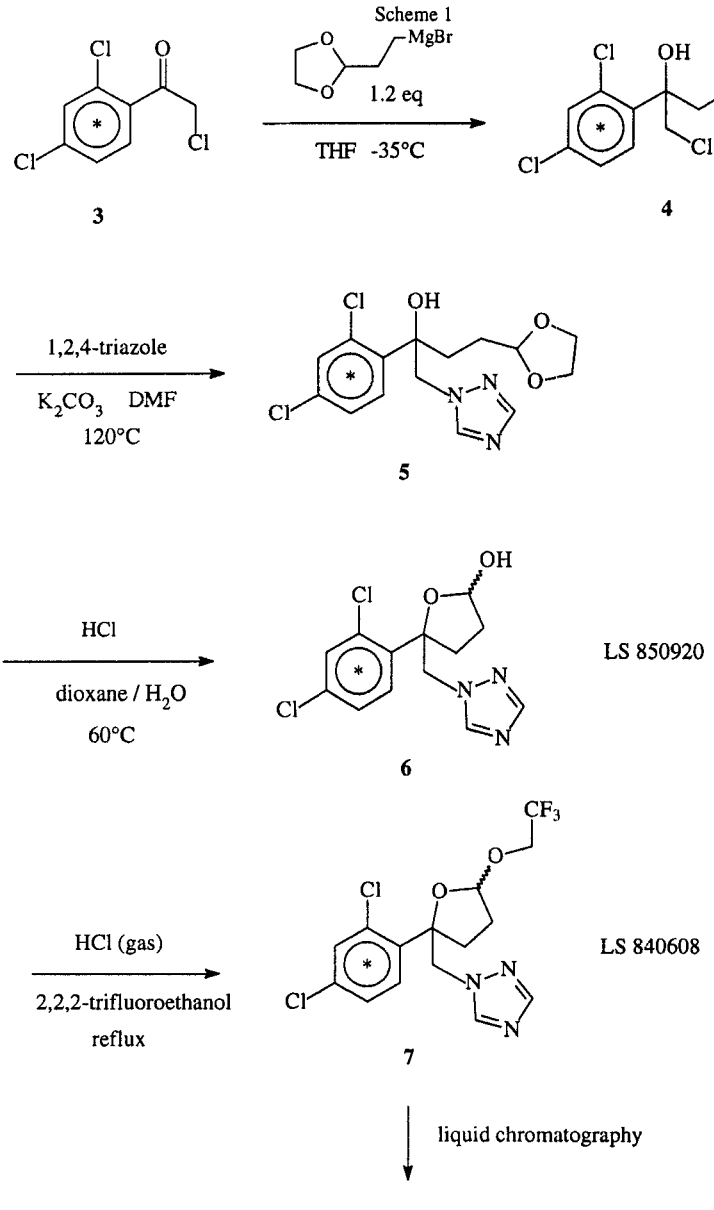
The structure of **1** is related to that of Propiconazole : **2** an agricultural fungicide [1b]. The present paper was under review when it came to our attention [1c] that Propiconazole labelled with ^{14}C in three different positions had been synthesized by E. Koltai and co-workers [1d].

CCC 0362–4803/95/121123–10

Received 13 March 1995
Revised 26 June 1995

DISCUSSION

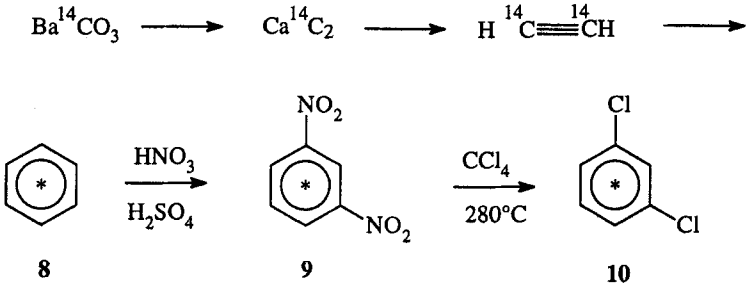
According to the industrial route (Scheme 1), the final radioactive product was obtained through 2,2',4'-trichloro-[ring U-^{14}C]acetophenone **3**, the key intermediate for this synthesis.



1 : [Phenyl U-^{14}C] LS 840606

1,3-Dichloro-[U-^{14}C]benzene **10** was first synthesized through 1,3-dinitro-[U-^{14}C]benzene **9** obtained by dinitration of [U-^{14}C]benzene **8** (2). The latter was prepared from [^{14}C] barium carbonate by the classical synthesis (Scheme 2). (3)

Scheme 2

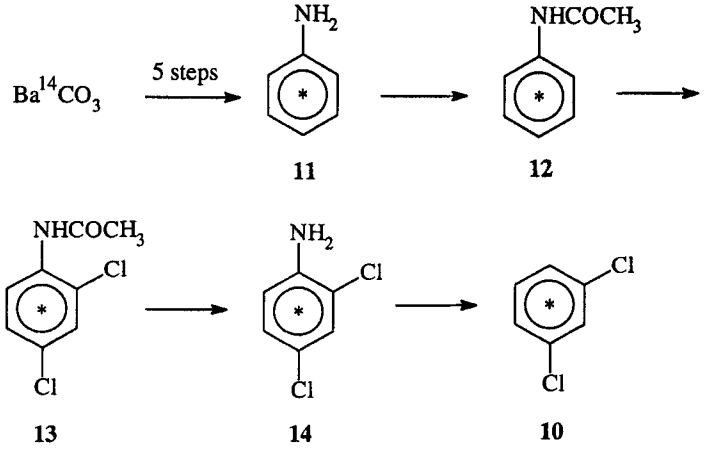


During a radioactive trial run the sealed tube containing 1,3-dichloro-[U-^{14}C]benzene broke in the steel bomb. To avoid the possibility of additional radioactive contamination, we chose a longer but safer alternative route previously used in our laboratory (Scheme 3).

1,3-Dichloro-[U-^{14}C]benzene **10** was obtained from [U-^{14}C]aniline **11** via [ring U-^{14}C]acetanilide **12**, 2,4-dichloro-[ring U-^{14}C]acetanilide **13** and 2,4-dichloro-[U-^{14}C]aniline **14**.

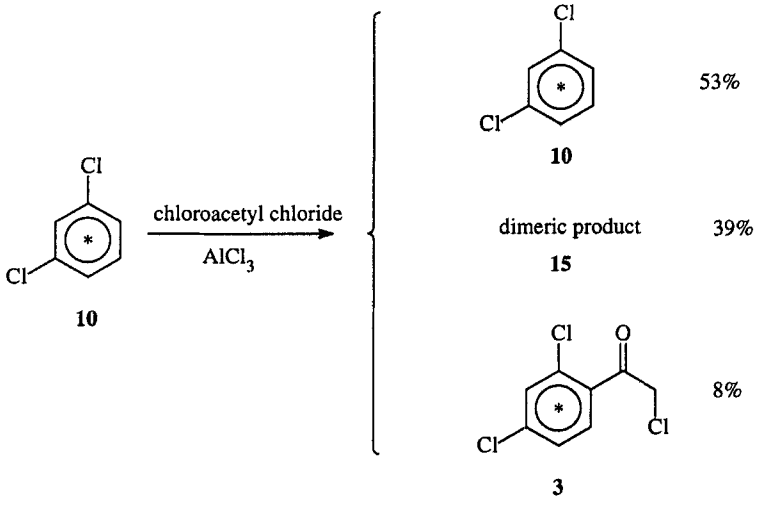
Compound **10** was synthesized from [U-^{14}C]aniline in 78% radiochemical yield and had a radiochemical purity of 93%.

Scheme 3



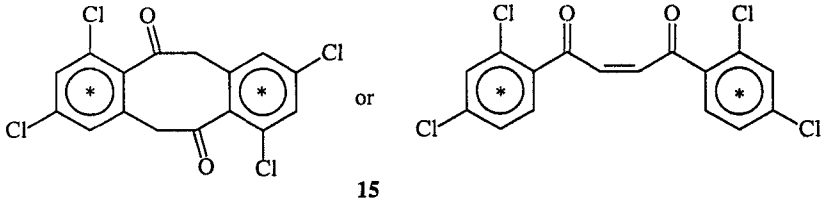
Preparation of **3** from unlabelled 1,3-dichlorobenzene proceeded in good yield (78%), in close agreement with its reported similar reaction with acetyl chloride to give 2,4-dichloroacetophenone. (4),(6)

Nevertheless radioactive results were very different with high specific activity. The Friedel-Crafts reaction gave two products, **3** and **15** as well as recovered **10**:



The expected product **3** was obtained in very low yield (8%), starting product **10** was recovered in 53% yield and by-product **15** was formed in significant yield (39%). After purification by liquid chromatography, this impurity was analyzed by mass spectrometry and a dimeric product structure was proposed (numerical simulations upon isotopic abundances gave fragment ion shapes in good agreement with the EI experimental spectra obtained, and we observed M=372 ($C_{16}H_8O_2Cl_4$), M-35, M-70, M-105 fragment ions).

Two structures were proposed for this dimeric product:



15

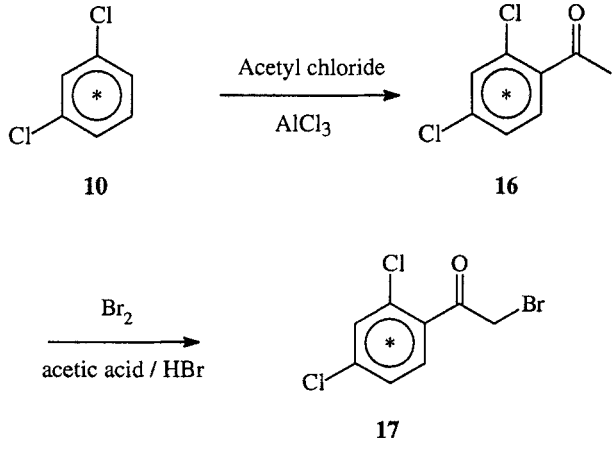
These assigned structures are only tentative since neither ^{1}H, ^{13}C-NMR spectra were recorded. Independant cold synthesis of the impurity for comparison of spectral data to absolutely confirm the structure were not undertaken.

This impurity could be due to a radioactive isotope effect. Some other examples are given in the literature for other reactions on [U-^{14}C]-ring labellings (5). The high radioactivity (50 mCi) used during the reaction in a small volume of solvent (2 mL) could be responsible for this unexpected product. However, yields for Friedel-Crafts reactions are sensitive to many variables, including reactant stoechiometries, reactant chemical and radiochemical purities, reaction temperatures, times and concentrations, the mode of reaction batch-up.

In the experimental section, there are enough differences in the procedures leading to **3** or **16** to suggest that some of these factors may also be involved in explaining the poor yield of **3** obtained.

We presumed also that the chlorine on the methyl group allowed for the formation of the impurity by intermolecular condensation. To circumvent formation of this undesired impurity, the synthetic approach was modified according to Scheme 4.

Scheme 4

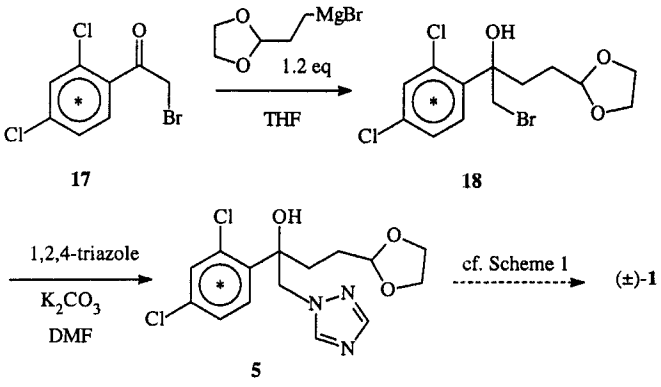


2',4'-Dichloro-[ring U-^{14}C]acetophenone **16** was obtained by reaction of acetyl chloride with **10**. (4),(6)

After purification by liquid chromatography a yield of 52% pure product was obtained. Further reaction of **16** with bromine in 1% HBr/acetic acid solution gave 2-bromo-2',4'-dichloro-[ring U-^{14}C]acetophenone **17** (7) in 55% yield after liquid chromatography purification. Unreacted **16** was brominated again in order to increase the total yield of this step to 80%.

Using **17**, target compound **1** was obtained as shown in Scheme 5.

Scheme 5



Condensation of 2,2-(ethylenedioxy)ethyl magnesium bromide with **17** gave 1-bromo-2-(2',4'-dichloro-[U-^{14}C]phenyl)-2-hydroxy-5-ethylenedioxypentane **18**. Reaction of 1,2,4-triazole with **18** gave 2-(2,4-dichloro-[U-^{14}C]phenyl)-5,5-(ethylenedioxy)-1-(1,2,4-triazol-1-yl)pentan-2-ol **5** which was cyclized to [phenyl U-^{14}C]-LS 850920 **4** (cf. Scheme 1). The two diastereomers of **7** were obtained by heating **6** with trifluoroethanol and gaseous hydrogen chloride and separated by medium pressure liquid chromatography.

EXPERIMENTAL

Mass spectra were obtained from a Varian CH7 apparatus. Reaction progress and radiochemical purities were determined by high performance liquid chromatography (HPLC) using a Dupont or a Waters chromatograph. Medium pressure chromatographic purifications were performed on Lichroprep Si60 40-63µ silica gel (Merck Shudart). Thin layer chromatography (TLC) was performed on Merck 60F 254 silica gel, or Whatman ODS-2 for reversed-phase TLC. Radioactivity was counted on a LKB 211 liquid scintillation counter.

2,2',4'-Trichloro-[ring U-^{14}C]acetophenone: 3

A mixture of freshly sublimed $AlCl_3$ (280 mg, 2 mmol), chloroacetyl chloride (0.145 mL, 1.8 mmol) and 1,3-dichloro-[U-^{14}C]benzene **10** (55 mCi/mmol, 50 mCi, 0.91 mmol) was heated at 50°C under a nitrogen atmosphere. The progress of the reaction was followed by HPLC.

Heating time	Starting product **10**	Expected product **3**	By-product **15**
2h	94%	5%	1%
4h	90%	7%	3%
22h	53%	8%	39%

After 22 h heating, the black mixture was hydrolyzed by pouring onto ice and extracted with dichloromethane (4x50 mL). The organic phase was washed with water, dilute sodium hydroxide, water and dried ($MgSO_4$). Total activity: 50 mCi.

[Ring U-^{14}C]acetanilide: 12

[U-^{14}C]Aniline hydrochloride (1400 mCi, 36 mCi/mmol, 30.8 mmol) was heated in carbon tetrachloride (43 mL) and acetic anhydride (12 mL) at reflux for 30 min. Additional acetic anhydride (12 mL) was added and heating was prolongated another 30 min. After evaporation of the solvents, the solid residue was refluxed with water (100 mL) for 1 h. The purity (99%) was checked by thin layer chromatography on silica gel , eluent: benzene: 50, ethyl acetate: 50. R_f: 0.55. Yield: 99%.

2,4-Dichloro-[U-^{14}C]aniline: 14

[Ring U-^{14}C]acetanilide **12** (4.16 g, 30.8 mmol, 1400 mCi) was dissolved in acetic acid (62 mL) containing fused sodium acetate (5.78 g, 70.5 mmol). Chlorine was bubbled through the solution until to reach an increase of weight of the theoretical amount (4.4 g, 62 mmol). After dilution with water (150 mL) the aqueous layer was extracted with ether (3 x 50 mL). The organic fractions were combined, washed with water (3x50 mL) and evaporated to dryness. The crude residual solid was then hydrolyzed with 2.5*N* HCl (240 mL) for 1.5 h at reflux.

The acidic aqueous phase was neutralised by addition of $NaHCO_3$ and then extracted with ether for 16 h in a liquid-liquid extractor. After evaporation of the solvent, the residual dark red oil was purified by medium pressure column chromatography (silica gel, eluent: benzene: 99, ethyl acetate: 1, and then on Whatman ODS-3 reversed-phase, eluent: methanol: 80, water: 20, triethylamine: 0.1). Yield: 63% of pure product (>99% checked by HPLC: Zorbax ODS, eluent: methanol: 80, water: 20, triethylamine: 0.1; k' = 4)

1,3-Dichloro-[U-^{14}C]benzene: 10

2,4-Dichloro-[U-^{14}C]aniline **14** (3.88 g, 24 mmol, 863 mCi) was diazotised at -5°C in 4*N* HCl (38 mL) with $NaNO_2$ (1.82 g, 26.4 mmol) dissolved in water (6 mL). After 30 min stirring at -5°C, the diazonium solution was dropped into a cold 50% aqueous hypophosphorous acid solution (22 mL). After evolution of nitrogen, ether was added (70 mL). The organic layer was dried ($MgSO_4$), the solvent evacuated at low temperature, and the product distilled under vacuum. A total of 803 mCi was obtained. The purity (93%) was checked by HPLC: Zorbax ODS, eluent: methanol: 80, water: 20, triethylamine: 0.1; k' = 5.8.

2,4-Dichloro-[ring U-^{14}C]acetophenone: 16

Aluminium trichloride (3.03 g, 22.8 mmol) was added to **10** (2.45 g, 16.7 mmol, 600 mCi) in a cylindrical flask. The suspension was heated to 50°C, and acetyl chloride (1.57 mL, 22.1 mmol) was then added dropwise. After heating at 110°C for 7.5 h, the thick black mixture was hydrolyzed with ice. The product was extracted with ether (150 mL), the organic layer washed with saturated aqueous $NaHCO_3$ solution (2x50 mL) and water (3x50 mL). After evaporation of the solvent, the black-brown oil was purified by column chromatography (Partisil ODS-3 , eluent: methanol: 80, water:

20). Concentration of the solution followed by extraction with ether afforded **16** (yield: 52%). Purity: 99% checked by HPLC: Zorbax ODS, eluent: methanol: 80, water: 20; k' = 3.5.

2-Bromo-2',4'-dichloro-[ring U-^{14}C]acetophenone: 17

A solution of bromine (1.28 g, 8 mmol) in acetic acid (1.5 mL) was added dropwise at room temperature to a solution of **16** (2.31 g, 12.2 mmol, 415 mCi) in acetic acid (4.6 mL) containing 1% of 48% hydrobromic acid. After 1.5 h stirring, the pale-yellow mixture was poured onto ice, extracted with ether (120 mL). The organic phase was washed with water (3x25 mL), saturated $NaHCO_3$ solution (3x25 mL), water (3x25 mL), and finally dried ($MgSO_4$).

Purification on silicagel (eluent: pentane: 98, ether: 2) gave 227 mCi of pure **17** (yield 55%). Unreacted starting product **16** was brominated again (final yield: 80%). TLC: (ODS phase): methanol: 80, water: 20; $R_f = 0.3$.

1-Bromo-2-(2,4-dichloro-[U-^{14}C]phenyl)-5,5-(ethylenedioxy)-pentan-2-ol: 18

A 0.62*M* solution of 2,2-(ethylenedioxy)ethylmagnesium bromide was prepared by reaction of freshly distilled 2-(2-bromoethyl)-1,3-dioxolane (4.53 g, 25 mmol) with magnesium (690 mg, 28 mmol) in dry THF (40 mL) at room temperature. The Grignard reagent (30 mL, 18.6 mmol) was then cooled to -45°C, and a solution of **17** (2.52 g, 9.4 mmol, 320 mCi) in THF (9 mL) was added dropwise. After 30 min stirring, acetic acid (1.9 mL) and water (20 mL) were added. The mixture was allowed to warm to ambient temperature and extracted with ether (20 mL). The colorless organic solution was washed with water and dried ($MgSO_4$). The purity (93%) was checked by TLC (silica gel, pentane: 97, ether: 3; $R_f = 0.15$).

2-(2',4'-Dichloro-[U-^{14}C]phenyl)-5,5-(ethylenedioxy)-1-(1,2,4-triazol-1-yl)-pentan-2-ol: 5

1,2,4-Triazole (1.3 g, 18.6 mmol) was added with stirring to a solution of **18** (3.48 g, 9.4 mmol, 320 mCi) in freshly distilled DMF (15 mL), followed by potassium carbonate (3.71 g, 26.8 mmol). The yellow mixture was heated for 15 h at 110°C. The solid residue was filtered and washed with DMF (4x30 mL). The solvent was evaporated under vacuum, the dark-red residual oil treated with water (50 mL) and a solution (1:1, v/v) of ethyl acetate and methylene chloride (50 mL). After drying the organic phase ($MgSO_4$), the purity was checked by TLC (silica gel, heptane: 45, ethyl acetate: 45, methanol: 10: 77% ; $R_f = 0.4$), (ODS phase, methanol: 80, water: 20: 80%; $R_f = 0.5$).

[U-^{14}C Phenyl] LS 850920: 6

Compound **3** (3.37 g, 9.4 mmol, 320 mCi) was dissolved in dioxane (17 mL). Water (43 mL) and concentrated hydrochloric acid (2.6 mL) were added. The mixture was then heated at 85°C for 4h. After evaporation of dioxane under vacuum, the aqueous phase was neutralized with dilute ammonia solution and extracted with methylene chloride. The organic phase was dried ($MgSO_4$) and evaporated. The solid residue was triturated with ether (60 mL), filtered, dissolved in methylene chloride and decolorized with charcoal. Total activity: 230 mCi, radiochemical yield: 72%. TLC: silica gel, dichloromethane: 45, ethyl acetate: 45, 2-propanol: 10, $R_f = 0.2$.

[U-^{14}C Phenyl] LS 840608: 7

The solution of **6** was evaporated, 2,2,2-trifluoroethanol added (12 mL, 167 mmol) and 5.2 mmol gaseous hydrogen chloride condensed by vacuum transfer. After warming to ambient temperature, the solution was heated at 80°C for 4h. A 5% (w/w) sodium carbonate solution (30 mL) was added, and the reaction mixture extracted with dichloromethane (2x60 mL). The organic phase was dried ($MgSO_4$). Total activity: 230 mCi. Radiochemical purity: 87%.

[U-^{14}C Phenyl] LS 840606: 1

The separation of diastereomers was performed by medium pressure chromatography on Lichroprep silica gel. Solvent: dichloromethane: 45, ethyl acetate: 45, 2-propanol: 10.

LS 840606 was first eluted. The solvent was evaporated, the solid residue dissolved in methanol and the solution treated with charcoal. Total activity: 99.6 mCi, specific activity: 37 mCi/mmol.

LS 840607 was then eluted. The solvent was evaporated, the solid residue dissolved in methanol and the solution treated with charcoal. Total activity: 93.8 mCi.

The purity was controlled by HPLC (Zorbax SIL, heptane: 75, ethyl acetate: 22, methanol: 3), k'LS 840606: 1.75, k'LS 840607: 6.8. Radiochemical purity: LS 840606: 99.4%, LS 840607: 96.4%

Analysis of LS 840606:

1) TLC (silica gel):

Heptane: 55, ethyl acetate: 40, methanol: 5. R_f 0.45, purity > 99%

2) TLC (ODS phase):

Methanol: 70, water: 30. R_f: 0.2, purity > 99%

3) HPLC:

Column: Zorbax Sil, mobile phase: heptane: 75, ethyl acetate: 22, methanol: 3

k' = 1.75, purity >99%

4) UV spectrophotometry in methanol.

λ_1 min: 244.5 nm, λ_2 min: 264.5 nm, λ_3 min: 274 nm

λ_1 max: 262.5 nm, λ_2 max: 269 nm, λ_3 max: 277.5 nm

5) Mass spectrometry (E. I.):

The mass spectrum was in agreement with the mass spectrum of an authentic sample.

The specific activity measured on the cluster 313-315-317-319 $\left(M^+ - \text{C}_3\text{H}_3\text{N}_2\text{-CH}_2 \right)$ was 37 mCi/mmol.

Acknowledgements

We thank Dr. Greiner from Rhône-Poulenc for fruitful discussions about this subject and the generous gift of unlabelled compounds.

REFERENCES

(1) Zech B., Gouot J.M., Merindol B. and Greiner A. - Brighton Crop Prot. Conf. -- Pests Dis. 503 (1988); Chem. Abst. 110: 130388 (1989).

(1b) The Merck Index - 11th Edition 1989. Merck and Co Inc. Rahway. N.J. USA n^r 7830.

(1c) Pichat L.- Co-editor of J. Labelled Compd. Radiopharm. - Private Communication.

(1d) Koltai E., Kling F., Rutkai G. - J. Labelled Compd. Radiopharm. 36 (1995) Accepted for publication.

(2) Moriya T. - J. Labelled Compd. Radiopharm. 14: 625 (1978).

(3) Noël J-P. and Pichat L. - J. Labelled Compd. Radiopharm. 13: 87 (1977).

(4) Schulze-Steinen H.J. and Falbe J.F. - Ger. Patent 1247289 (1967); Chem.Abst. 68: 77980 (1968).

(5) Parker D. G. - In Synthesis and Applications of Isotopically Labelled Compounds 1985; Muccino R.R., Ed.; Elsevier Science: Amsterdam, 1986; pp 491-496.

(6) Leonard N.J. and Boyd S.N. Jr - J. Org. Chem. 11: 405 (1946).

(7) Buu-Hoï Ng. Ph., Xuong Ng. D. and Khoï Ng. H. - J. Chem. Soc. 255 (1951).

(8) Kornblum N. - In Organic Reactions; Adams R., Ed.; John Wiley & Sons; New York, NY, 1944; Vol 2, Chapter 7, p 294.