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NOTE

[^{13}C, $^{2}H_4$]MELATONIN

D. Le Bars[1], C. Luu-Duc[2], A. Marsura[2], J. Chantegrel[2]

[1] Centre d'Exploration et de Recherche Médicales par Emission de Positons (CERMEP) 59, Bd Pinel 69003 Lyon, France.

[2] URA C.N.R.S. n° 214, Laboratoire de Chimie-Pharmacie, UFR de Pharmacie, Université Joseph Fourier Grenoble I, 38700 La Tronche, France.

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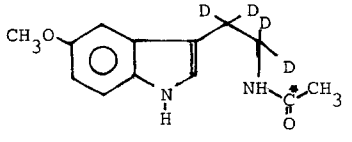
INTRODUCTION

Labelled acetyl chloride was chosen to synthesise [^{13}C, $^{2}H_4$]melatonin from tetradeuterated methoxytryptamine. In our previous work, acetylchloride was labelled with the short-lived isotope carbon-11 to produce [^{11}C]-melatonin and [^{11}C]-6-fluoromelatonin for Positron Emission Tomography (1, 2). With minor adjustments, it was possible to replace [^{11}C] carbon dioxide by [^{13}C] carbon dioxide. [^{13}C] n.m.r spectra of the title compound was performed, giving unequivocal data (3).



EXPERIMENTAL

5-Methoxy tryptamine base and melatonin were purchased from Sigma Chemical Co. Ltd. Phthaloyl dichloride and 2,6-di-tert-butylpyridine were obtained from The Aldrich Chemical Co. Ltd. and were distilled under nitrogen before use. Diethyl ether (Analar Grade, BDH Chemicals Ltd) was dried over sodium. All other solvents were purchased from BDH Chemicals Ltd.

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or from Fisons Ltd. and were either of Analar or HPLC grade. Magnesium turnings, from BDH Chemicals Ltd, were kept in an oven at 120°C before use. Nitrogen (oxygen-free) was obtained from BOC Ltd and dried over magnesium perchlorate, BDH Chemicals Ltd. Enriched potassium [^{13}C] carbonate (91 atom %) was obtained from Amersham International.

Methylmagnesium bromide

Methylmagnesium bromide (0.5 M) in diethyl ether was freshly prepared by the technique described previously (4).

Tetradeuterated [^{13}C] Melatonin

Carbon-13 enriched carbon dioxide (91 atom %) was generated by adding sulfuric acid on [^{13}C] potassium carbonate (2 mg, 15 µmoles). The gas was allowed to bubble in 200 µl of freshly prepared methylmagnesium bromide (0.5 M) in ether, carried with a flow of nitrogen (5 ml/min). After 2 minutes, the carbonation was quenched by phthaloyl dichloride (100 µl, 0.7 µmol) plus 2,6-di-tert-butylpyridine (200 µl, 0.85 mmol). The mixture was then heated with hot air to 80°C and the flow of nitrogen increased to 25 ml/min to distill off [^{13}C] acetylchloride (bp 52°C) into cold dichloromethane (1 ml, 0°C) containing tetradeuterated 5-methoxytryptamine base (5mg, 26 µmol) (5).

After 10 minutes of distillation, the resulting suspension was loaded through a filter (Acrodise, Gelman Ltd) to a silica column (30 cm x 0.7 cm i.d.; µ-Porasil, Waters Associated) eluted at 4 ml/min with dichloromethane containing a mixture of ethanol, water and triethylamine (100:2:2, v:v:v). The eluant was monitored for absorbance at 278 nm, and the fraction having the same retention time as reference melatonin (5 min 40 s) was collected and evaporated to dryness. The starting material in excess, tetradeutero-5-methoxytryptamine elutes later (retention time: 12 min) and is recycled. The response of the absorbance detected at 278 nm was calibrated with known amounts of melatonin, in order to calculate directly the quantitities synthesised. A typical run yields 300 µg of tetradeutero[carbonyl-^{13}C] melatonin (1.3 µmol), i.e. a yield of 5% from the starting amine and 8.6% from the [^{13}C] carbon dioxide. TLC analysis using silica plates (Camlabs), eluted with ethylacetate or chloroform:ethanol (9:11, v:v) reveal only one compound (Rf: 0.18 and 0.52), co-migrating with authentic melatonin sample.

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