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Note

Dithizone-^{14}C labelling and characterization

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Summary

[Thiocarbonyl-^{14}C] Dithizone was prepared and characterized spectroscopically. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: dithizone; carbon-14

Discussion

Dithizone **1** was first synthesized by renowned chemist Emil Fischer in 1878,[1] while its definitive structural elucidation awaited for decades the work of Corwin[2] along with a refined crystal determination by Laing.[3] Hutton later explored the issue of dithizone tautomers in various solvents.[4] The compound has had widespread use as an analytical reagent but we were required to label it with ^{14}C for a biological application. To our knowledge the preparation of [^{14}C] dithizone has not been described, but the literature does report its labelling with ^{13}C, modelled after the original work of Fischer.[4] However, we had difficulty in directly applying this particular method to the task of ^{14}C labelling. We were successful though in utilizing an *Organic Synthesis* procedure[5] with purification modification and now report the first preparation of [thiocarbonyl-^{14}C] dithizone **2**.

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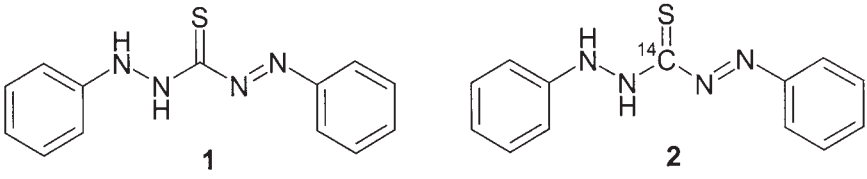
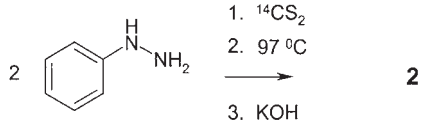


1 **2**

Scheme 1 summarizes the conversion of [^{14}C] carbon disulfide[6] to [thiocarbonyl-^{14}C] dithizone **2**. Surprisingly, the *Organic Synthesis* procedure employed only an extraction purification with minimal purity and identity confirmation. We decided to chromatographically purify and fully characterize **2** to the extent possible. We could locate no report of a robust HPLC system to characterize **1** and although **2** was purified by silica gel flash chromatography, we also were not successful in developing a reproducible analytical HPLC protocol for it by either reverse or straight phase methodology. However, convincing spectroscopic evidence corroborated the identity and radiopurity of **2**. The proton NMR ($CDCl_3$) of **2** matched that of authentic **1** and its mass spectrum displayed a prominent molecular ion with a characteristic fragmentation pattern similar to **1**, but offset higher by two mass units. Equally compelling was the unique UV-visible spectrum ($CHCl_3$) of **2** which was completely superimposable on that of **1** and provided a specific activity value of 55 mCi/mmol, in close agreement with the value of 53 mCi/mmol for **2** derived independently by mass spectral analysis. The approach we report here could also be adapted for the preparation of [ring-^{14}C] dithizone *via* [^{14}C] phenylhydrazine.



Scheme 1. Synthesis of 2

Experimental

Evaporations were carried out on a Buchi rotary evaporator at bath temperatures less than 40°C. All chemicals used were reagent grade and authentic **1** was purchased from Aldrich. The proton NMR spectra were obtained on a Bruker 300 MHz instrument and chemical shifts are reported in parts per million (ppm) downfield from TMS. The mass

spectra were obtained on a Finnigan LC Deca instrument with direct injection.

[Thiocarbonyl-^{14}C] diphenylthiocarbazide

To a solution of 794 mg (7.34 mmol) of phenylhydrazine in 3 ml of diethyl ether in a 100 ml round bottom flask fitted with a water cooled condenser was added 251 mCi (4.74 mmol, 53 mCi/mmol) of [^{14}C] carbon disulfide[6] in 0.4 ml of diethyl ether. The mixture was stirred for 0.5 h, resulting in a precipitate that was washed with 0.5 ml of diethyl ether affording 217 mCi (1.2 g, 4.1 mmol, 86.5% radiochemical yield) of [thiocarbonyl-^{14}C] beta-phenyldithiocarbazic acid, phenylhydrazine salt. A portion of this salt (43.5 mCi, 240 mg, 0.82 mmol) was transferred to a 25 ml round bottom flask and heated at 97°C for 40 min and the solid softened to a yellow–green foam. After cooling to 0°C, 0.2 ml of ethanol was added and the slurry was allowed to sit at ambient temperature for 1 h. The resulting solid was filtered and washed with 0.1 ml of ethanol yielding 33.9 mCi (166 mg, 0.64 mmol, 78% radiochemical yield) of crude [thiocarbonyl-^{14}C] diphenylthiocarbazide that was used directly for the next step.

[Thiocarbonyl-^{14}C] dithizone (**2**)

The above intermediate was added to a solution of 112 mg of potassium hydroxide in 1 ml of methanol and the flask was heated to reflux for 5 min. The resulting red solution was cooled in an ice bath and 2 ml of ice cold 1 N sulfuric acid was added, bringing the pH to 3. The product was extracted into 10 ml of chloroform, dried over sodium sulfate, filtered and evaporated to a volume of 5 ml and applied to a silica gel (Fluka, 270–440 mesh) flash column eluted with chloroform. The deep green fractions emerging from the column were combined and evaporated to afford 6.36 mCi (30 mg, 0.12 mmol, 18.8% radiochemical yield) of product **2**; proton NMR ($CDCl_3$) δ 7.65 (m, 4), 7.45 (m, 4) and 7.30–7.20 ppm (m, 2); mass spectrum *m*/*e* 259 (MH^+) with a measured specific activity of 53 mCi/mmol; UV-Visible ($CHCl_3$) 440, 610 nm with a measured specific activity of 55 mCi/mmol.

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