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Synthesis of Caffeine-2-^{14}C

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SUMMARY

Xanthine-2-^{14}C (**1**) *was converted to caffeine-2-^{14}C* (**2**) *by methylation with dimethyl sulfate.*

INTRODUCTION.

In connection with a research project on the metabolism of caffeine (**2**) by micro-organisms [1] radioactive caffeine was needed. The only commercially available ^{14}C-labelled caffeine is caffeine-1-methyl-^{14}C *. However, ring labelled caffeine would be preferable for metabolic studies, because the N-methyl group may be lost early in the course of caffeine degradation. It was decided, therefore, to prepare caffeine-2-^{14}C by methylation of the commercially available xanthine-2-^{14}C ** (**1**). The method chosen for this synthesis is an adaptation of the procedure described by Bredereck *et al.* [2].

EXPERIMENTAL

Xanthine-2-^{14}C, having a specific activity of 50 μC per 158 μg, was dissolved in 0.25 *N* sodium hydroxide to give a solution with 1 μC/ml. In a fume hood a 100-ml beaker, containing 25 ml of this solution, 15 ml of water, and 2 ml of dimethyl sulfate ***, was mounted on a magnetic stirrer. The electrodes of a pH meter were introduced into the mixture and 5 *N* sodium hydroxide was added dropwise with continuous stirring at such a rate that the

* Tracerlab, Irvine, California. Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

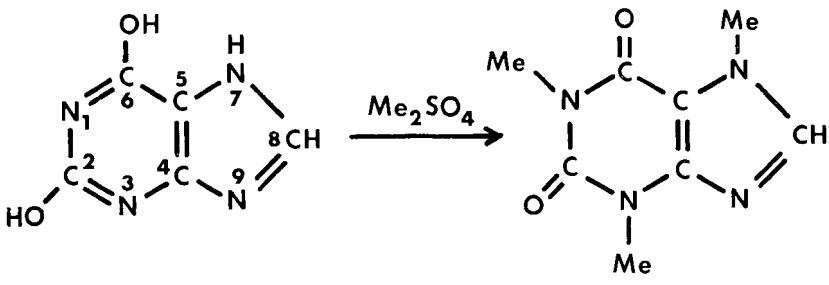
** Schwarz Bioresearch, Inc., Orangeburg, New York.

*** Pfaltz and Bauer, Inc., Flushing, New York, CARE, EXTREMELY TOXIC.

pH remained near 9. During the first 15 min very little sodium hydroxide was required. After about 2½ hr, the reaction was completed.

The reaction mixture was acidified to pH 4.0 with 0.1 *N* hydrochloric acid, transferred to a separatory funnel, and extracted with five 10-ml portions of chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate. The solvent was removed under vacuum in a rotary evaporator and the residue was taken up in 25 ml of ethanol.

The radioactivity of this solution was determined with a liquid scintillation counter [+] in a scintillation solution cotaining 6 g 2,5-diphenyloxazole (PPO) [++] and 150 mg 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) [++] per liter of toluene [+++]. Based on the radioactivity of the xanthine-2-^{14}C solution, determined in 1 *M* Hyamine Hydroxide [++] in methanol, the yield from this preparation was 72.6%.



XANTHINE **CAFFEINE**

1 **2**

The identity of the product was established by application of the thin-layer chromatographic method of Heftmann and Schwimmer [3]. Samples of the product and of authentic caffeine were applied to 5 × 20-cm glass plates, precoated with a 0.25-mm layer of Silica Gel GF *. The plates were developed in each of three solvent systems : chloroform-methanol (4 : 1), ethyl acetate-methanol-ammonium hydroxide (8 : 1 : 1), and *n*-butanol, saturated with a 2.8% aqueous solution of ammonium hydroxide. The thin-layer chromatograms were scanned in a radiochromatogram scanner ** and viewed in short-

[+] Tri-Carb Liquid Scintillation Spectrometer, Model 3003, Packard Instrument Co., Inc., LaGrange, Illinois.

[++] Amersham/Searle, Des Plaines, Illinois.

[+++] Spectroquality. Matheson Coleman and Bell, Los Angeles, California.

* Uniplates. Analtech, Inc., Newark, Delaware.

** Packard Radiochromatogram Scanner, Model 7201. Packard Instrument Co., Inc., LaGrange, Illinois.

wave (254 mμ) ultraviolet light ***. In each solvent system the peak of radioactivity coincided with the location of the quenching of background fluorescence. The radiochromatograms showed no evidence of impurities in excess of 2%. The starting material, xanthine-2-^{14}C, when similarly chromatographed, also travelled as a single radioactive zone of at least 98% purity.

REFERENCES

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3. HEFTMANN, E. and SCHWIMMER, S. — *J. Chromatog.*, **59** : 214 (1971).

*** Chromato-Vue Cabinet. Ultra-Violet Products, Inc., San Gabriel, California.