

## SYNTHESIS OF $[2-^{14}\text{C}]$ THEOBROMINE

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### SUMMARY

$[2-^{14}\text{C}]$ theobromine was prepared by the methylation of commercially available  $[2-^{14}\text{C}]$ xanthine with dimethyl sulfate. The yield was 44.5 % of the initially applied radioactivity.

### INTRODUCTION

Theobromine (3,7-dimethylxanthine) is often found in nature as an accompanying substance of caffeine, for instance, in the well known stimulants, such as tea, coffee, cocoa and cola. The simultaneous occurrence of these two purine alkaloids is explained by their biosynthesis: Theobromine is the direct precursor of caffeine (1,2,3). In spite of the universal nature of theobromine, there are only few publications dealing with its physiology and pharmacology. A prerequisite to this type of study is the availability of theobromine as ring labelled tracer.

Methods of chemical synthesis have been described several times (4,5,6) but they are badly suitable in the production of ring labelled theobromine. 1976 Ayrey and Yeomans (7) obtained a low yield of  $[2-^{14}\text{C}]$ theobromine by methylation of  $[2-^{14}\text{C}]$ xanthine with dimethyl sulfate. We have modified this procedure, aiming at an optimal output of theobromine.

## EXPERIMENTAL

In a preliminary experiment using 0.2 mg xanthine and 2  $\mu$ Ci  $[2-^{14}\text{C}]$ xanthine (Commissariat de l'Energie Atomique, Paris, France), the yield of theobromine as regards to the added dimethyl sulfate was studied. Xanthine was dissolved in 10 ml buffer pH 9 (0.05 m sodium borate with 0.05 m KCl). Every 4 hours 20  $\mu$ l of dimethyl sulfate were pipetted into the reaction mixture, which was constantly stirred. The reaction took place at room temperature, and the pH was maintained at 9 by addition of 3N KOH (pH-stat E 473, Metrohm, Herisau, Switzerland). Before every new addition of dimethyl sulfate, a 20  $\mu$ l sample was chromatographed on TL, Silica gel F 254 (Merck, Darmstadt, Germany) in solvent system 1 (chloroform-methanol, 19 : 1) and the actual distribution of radioactivity was measured by radiochromatogram scanning. Co-chromatographed reference substances were theobromine ( $R_F$  0.24), theophylline ( $R_F$  0.28) (both from Sigma, St. Louis, USA), caffeine ( $R_F$  0.46), paraxanthine ( $R_F$  0.21), 1-methylxanthine ( $R_F$  0.08), 3-methylxanthine ( $R_F$  0.10), 7-methylxanthine ( $R_F$  0.08), xanthine ( $R_F$  0.00) (all from Fluka, Buchs, Switzerland).

Fig. 1 shows the kinetics of theobromine and caffeine formation. The so-called rest activity consists of xanthine and unidentified substances with  $R_F$  values lower than 0.10. Optimal yield of theobromine is reached after the 3rd or 4th addition of dimethyl sulfate. In the main experiment, 20  $\mu$ l of dimethyl sulfate were added 4 times to 80  $\mu$ Ci of  $[2-^{14}\text{C}]$ xanthine in 10 ml buffer. At the end of the reaction, samples of 20  $\mu$ l were chromatographed with solvent system 1 and with ethyl acetate-methanol-0.9l ammonia (8 : 2 : 1; solvent system 2). The  $R_F$  values were 0.44 (theobromine), 0.59 (caffeine), 0.26 (paraxanthine),

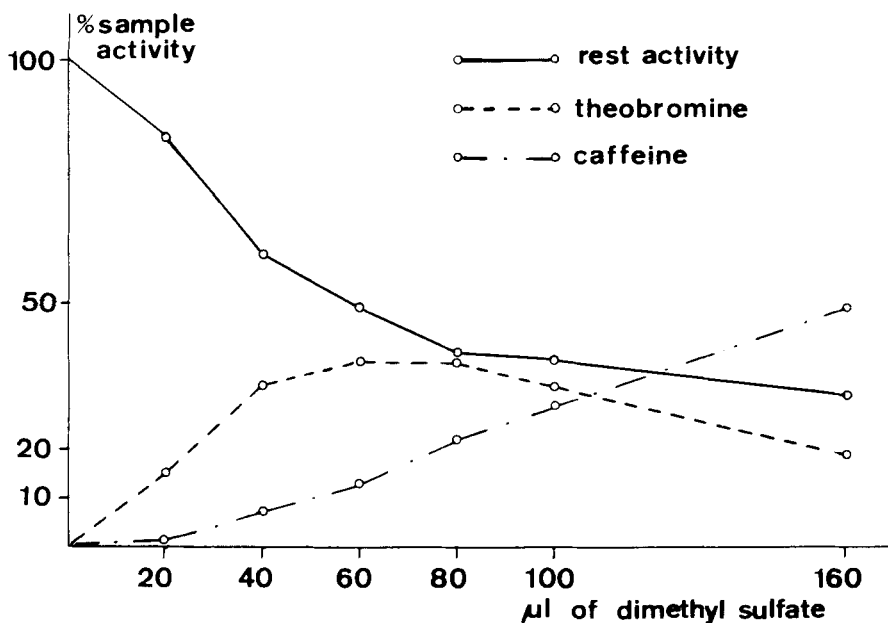


Fig. 1 Alteration in radioactivities in a reaction mixture of 0.2 mg xanthine, 2  $\mu\text{Ci}$   $[2-^{14}\text{C}]$  xanthine, pH 9, 25° C, with 20  $\mu\text{l}$  of dimethyl sulfate every 4 hours added.

0.23 (theophylline), 0.10 (1-methylxanthine), 0.15 (3-methylxanthine), 0.11 (7-methylxanthine), 0.04 (xanthine). Radiochromatogram scanning gave the following relative distribution of radioactivity: Theobromine 49%, caffeine 26%, rest activity 25%. The reaction mixture was filled up with water to 20 ml and applied into a diatomaceous earth column (8), (160 x 25 mm; 11.5 g Extrelut, Merck, Darmstadt, Germany), which was eluted with 4 x 40 ml of chloroform. After the evaporation of the solvent, the residue was redissolved in 1 ml of chloroform and finally applied on a TL plate (solvent system 2). Theobromine and caffeine were eluted with water from the thin layer. The following yields were

obtained: Theobromine 36.5  $\mu\text{Ci}$ , corresponding to 44.5% of the initially applied radioactivity and caffeine 19.5  $\mu\text{Ci}$  or 23.5%. Identity of theobromine was examined by HPLC (Waters,  $\mu\text{Bondapak C}_{18}$ , water-methanol 3 : 1), UV-spectrum and co-chromatography in solvent systems 1 and 2. Radiochemical purity, tested by chromatogram scanning (solvent system 1), was greater than 99%.

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