SYNTHESIS OF [2-^{14}C] TETRAMETHYLURIC ACIDS

P.S. Citroreksoko*, J. Petermann, H. Wanner and T.W. Baumann**
Institute of Plant Biology, University of Zurich, Switzerland

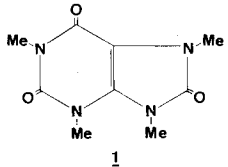
SUMMARY

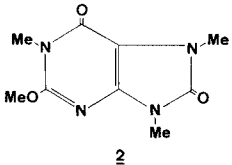
[2-^{14}C]1,3,7,9-, 0(2),1,7,9- and 0(8),1,3,7-Tetramethyluric acid were prepared by methylation of [2-^{14}C]uric acid with dimethyl sulfate at pH 9. The yield after repeated (3x) purification by TLC was 44.9%, 4.4% and 6.3% respectively.

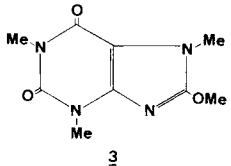
Key word: Methyluric acids

INTRODUCTION

In 1937 Johnson (1) reported the existence of a new purine in nature. From the residue left after the commercial extraction of caffeine from "several million pounds" of tea he isolated hexagonal crystals which he identified as 1,3,7,9-tetramethyluric acid (1,3,7,9-TMUA, 1). This report remained unconfirmed until recently when Wanner et al. (2) detected this methylated oxypurine together


1


2


3

with a trimethyluric acid in leaves of some Coffea species collectively designated as "liberio-excelsoids" (3). Shortly after that a second tetramethyluric acid was found in those leaves and identified by Petermann et al. (4) as 0(2),1,7,9-tetramethyluric acid

---

*Present address: National Biological Institute, Indonesian Institure of Sciences, Bogor, Indonesia

**To whom the correspondence should be addressed

0362-4803/80/0617-0775$01.00 Received June 29, 1979

(0(2),1,7,9-TMUA, 2). Detailed analyses (5) of the liberio-excelsoid species finally revealed that the seeds used for coffee by natives of Westafrica also contain these substances as well as a third tetramethyluric acid, namely the 0(8),1,3,7-tetramethyluric acid (0(8),1,3,7-TMUA, 3).

Substance 1 is a highly efficient solubilizer of many carcinogenic compounds such as polycyclic aromatic hydrocarbons (6). Its cytogenetical effects have been reviewed recently by Kihlman (7). As a comparison, the solubilizing power of substance 3 is lower, but its ability to produce chromosomal aberrations is higher (6,7). Substance 2 has never been tested in this respect.

To study the mode of action of these tetramethyluric acids on the genetical material and to elucidate their metabolic fate in caffeine-containing plants or in mammals, ring-labelled compounds should be at one's disposal. A simple synthesis of these naturally occurring substances is described, which is based on classical preparations of this class of compounds.

EXPERIMENTAL

Synthesis. To 10 μCi of [2-^{14}C]uric acid with a specific activity of 51 mCi/mmol (The Radiochemical Centre, Amersham, England), dissolved in 10 ml H_2O, 2 ml of freshly distilled dimethyl sulfate were added. The pH of the constantly stirred reaction mixture was maintained around 9.0 by adding 2 N NaOH (pH-stat, Metrohm, Herisau, Switzerland). After completion of the reaction (22 hr) the solution was acidified (pH 4.0) with 0.1 N HCl and extracted with $CHCl_3$ (4 x 10 ml). The $CHCl_3$ phases were combined and $CHCl_3$ was evaporated.

Purification. The residue was dissolved in $CHCl_3$ and chromatographed on TL (SiO_2 gel 60 F_{254}, Merck, Darmstadt, Germany) with

the following solvents in this order: 1) $CHCl_3$/MeOH 19:1, 2) acetone/benzene 7:3 (chamber satd with ammonia), 3) $CHCl_3$/MeOH 9:1. Authentic samples of 1, 2 and 3 served as reference substances (2). Radioactivity distribution on the TL was determined with a radiochromatogram scanner (Berthold, Karlsruhe, Germany). Radioactive areas were cut out and eluted with MeOH (5 ml). Aliquots of 10 µl were taken for scintillation counting in 10 ml Bray's solution (8). For rechromatography the eluate was dried and redissolved in $CHCl_3$. To obtain UV spectra for product identification a parallel "cold" synthesis was made.

RESULTS

In the combined chloroform phases 83.7 % of the initial radioactivity were present with substance 1, 2 and 3 as major products. Yields and R_f values are listed in Table 1.

Table 1: [2-^{14}C]Tetramethyluric acids. Yields and R_f values after sequential chromatographic separations. The R_f values in solvent 2 were 0.43, 0.52 and 0.60, respectively. Radioactivity of uric acid was 22 x 10^6 dpm.

Product	1. Chromatogram (Solvent 1)		2. Chromatogram (Solvent 3)	
	Yield (%)	R_f	Yield (%)	R_f
1,3,7,9-TMUA	56.8	0.33	43.9	0.48
0(2),1,7,9-TMUA	10.0	0.48	4.4	0.57
0(8),1,3,7-TMUA	9.1	0.57	6.3	0.71

There were at least 4 substances amounting totally to 8 % of the initial radioactivity with R_f values lower than 0.28. No attemps were made to identify them. Radiochemical purity of all

three methyluric acids was greater than 95 % after the third chromatographic run.

ACKNOWLEDGEMENTS

We were financially assisted in this work by the Swiss National Science Foundation. We thank Dr. Vera C. Klein for correcting the English text and Heidi Müller for secretarial help.

REFERENCES

1. Johnson, T.B. - J. Amer. Chem. Soc. 59: 1261 (1937)
2. Wanner H., Pesakova M., Baumann T.W., Charubala R., Guggisberg A., Hesse M. and Schmid H. - Phytochemistry 14: 747 (1975)
3. Charrier A. and Berthaud J. - Café Cacao Thé 19: 251 (1975)
4. Petermann J., Baumann T.W. and Wanner H. - Phytochemistry 16: 620 (1977)
5. Citroreksoko P.S. - Thesis University of Zurich, 1979
6. Weil-Malherbe H. - Biochem. J. 40: 351 (1946)
7. Kihlman B.A. - Mutation Res. 39: 297 (1977)
8. Bray G.A. - Anal. Bioch. 1: 279 (1960)