Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXIV, No. 3

NOTE

SYNTHESIS OF U-^{14}C-LABELLED 5-HYDROXYMETHYL-2-FURALDEHYDE

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SUMMARY

Uniformally ^{14}C-labelled 5-hydroxymethyl-2-furaldehyde was synthesized by dehydration of D-[U-^{14}C]fructose on an H^+ ion exchange resin and a water isobutylmethyl ketone biphasic liquid reaction medium. The optimal reaction time was 45min, and after HPLC purification the radiochemical yield was 50%. The whole process took 2 hours.

Keywords:dehydration, ion exchange resin, [U-^{14}C]5-hydroxymethyl-2-furaldehyde

INTRODUCTION

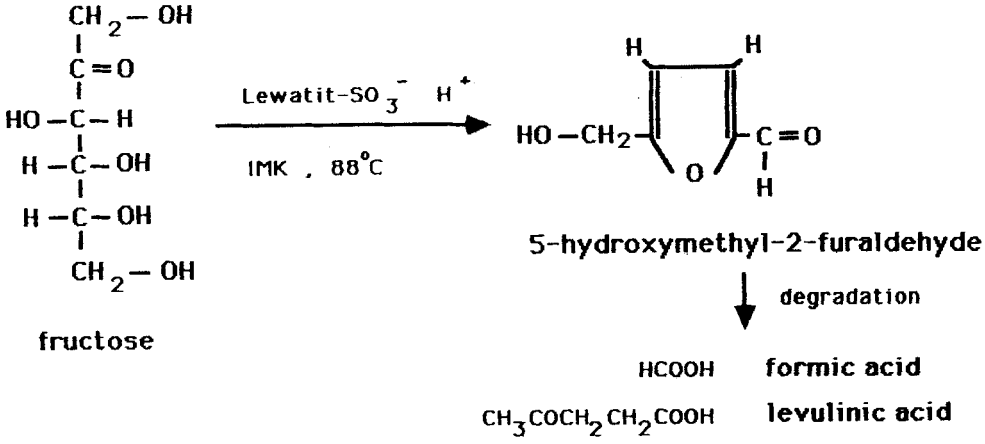
5-Hydroxymethyl-2-furaldehyde (HMF) is a Maillard intermediate in the degradation of hexoses in acidic media. Its presence in food is often reported, for example in milk where the content of HMF was found to increase during heat treatment (1). It can also be found in parenteral solutions, where it can be generated during sterilization at low pH (2,3). In addition, HMF reduces the nutritional value of food by reacting with lysine (4). It was thus important to synthesize radiolabelled HMF to study its metabolism, its distribution and that of its metabolites in the animal, and also its chemical interaction with food constituents. This paper presents a rapid and efficient single-step preparation of [U-^{14}C]HMF starting from D-[U-^{14}C]fructose, which is a microscale

0362-4803/87/030343–06$05.00

Received March 17, 1986

modification of the synthetic procedure described by Rigal et al (5). The synthesis of HMF involves the catalytic dehydration of D-fructose with an H^+ ion exchange resin (see Scheme 1). A water-isobutylmethylketone biphasic liquid reaction medium is used to accumulate HMF in the organic phase as it is formed. This procedure minimises degradation of HMF into levulinic acid and formic acid.

Scheme 1 : Dehydration of D-Fructose



RESULTS AND CONCLUSION

Rigal et al. (5) synthetised HMF by dehydrating fructose on an ion exchange resin. A yield of 50% was attained when 2.2g of fructose in 100ml reaction mixture was reacted at 88°C for 15hrs. However, the synthesis of labelled compounds with high specific radioactivity implies the use of microquantities of chemicals in very small volumes, where optimal reaction conditions often differ from those of larger scale synthesis. The optimal microscale reaction time was determined by following the reaction of 1µCi of D-[U-^{14}C]fructose along with 275µg of cold D-fructose in 0.5ml of water-solvent mixture and in the presence of the H^+ ion exchange resin. The reaction temperature was maintained at 88°C. Fractions of the organic phase were taken every 10min and analysed by TLC. In our conditions, HMF migrated with an Rf of 0.59 and

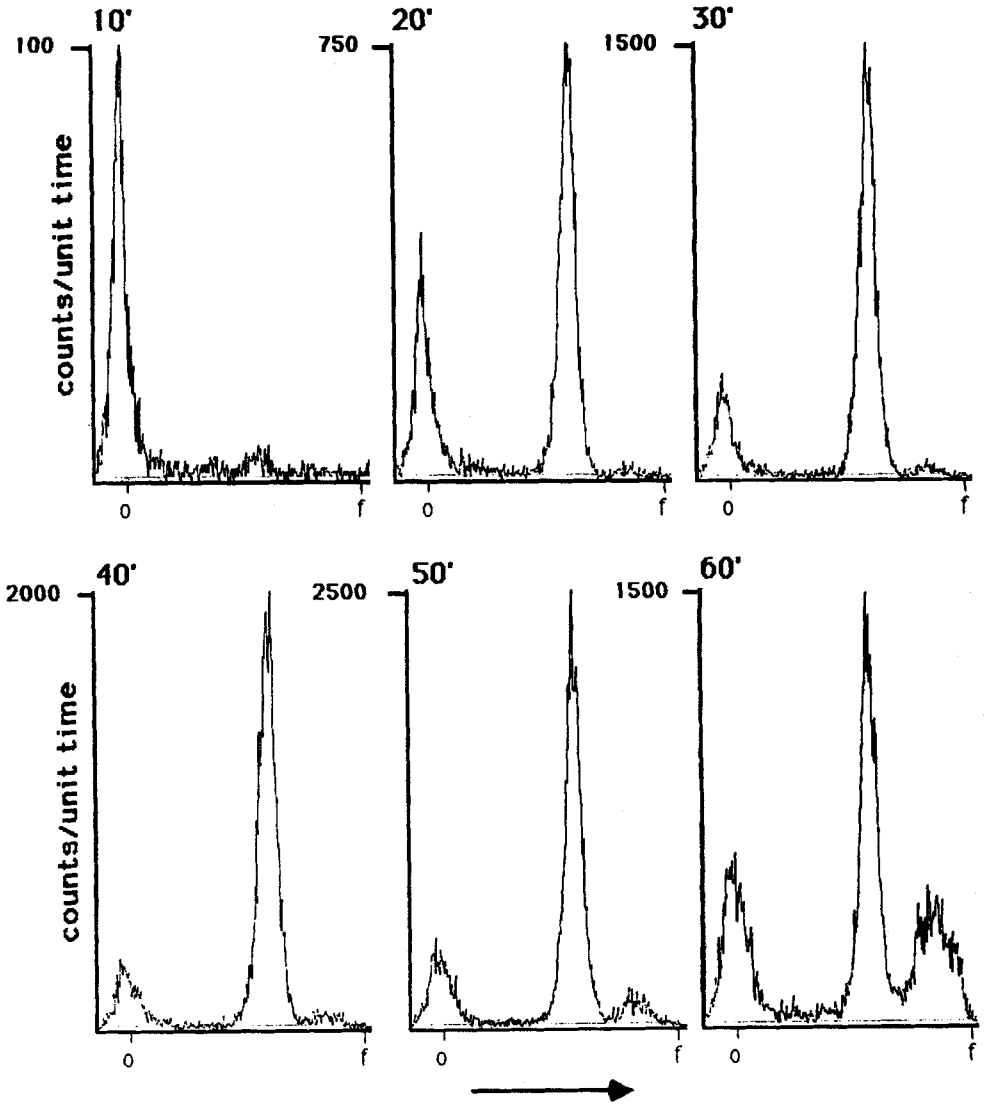


FIGURE 1 : TLC radiochromatograms obtained during the synthesis of HMF (o: origin, f: front; counts are recorded over the same time period for each time point).

fructose remained at the origin. Figure 1 shows that after 10min, the fructose, which is poorly soluble in the solvent, is detected along with trace amounts of HMF. Then HMF progressively appears, becoming after 40min the main radiolabelled product. Prolonged reaction time led to the appearance of degradation products some migrating with an Rf of 0.86 and others like fructose

remaining at the origin of the chromatogram. The identification of these products was not attempted.

The synthesis of [^{14}C]HMF with high specific radioactivity was done by replacing part of the cold fructose by 200µCi of D-[U-^{14}C]fructose to maintain the same initial fructose concentration. The reaction mixture was held at 88°C for 45min, then rapidly chilled. HMF was recovered by evaporation of the solvent phase under vacuum and purified by HPLC. Figure 2 shows a TLC analysis of the purified [^{14}C]HMF which is over 98% radiochemically pure. The overall radiochemical yield from starting D-[U-^{14}C]fructose is 50%. It is interesting to note that for a 40-fold reduction of the fructose concentration compared to Rigal's conditions, a similar reaction yield is attained after 40min compared to 15 hrs.

This study describes a rapid and convenient synthesis of [^{14}C]HMF from a commercially available substrate, the D-[U-^{14}C]fructose. This synthesis could also be used to obtain HMF labelled with a stable isotope such as ^{13}C in order to extrapolate the metabolic results obtained in the animal to man.

EXPERIMENTAL

Materials :

D-fructose and isobutylmethylketone (IMK) were from Merck (Darmstadt, D). D-[U^{14}C]fructose (274 mCi/mmole, 10.14 GBq/mmol) was obtained from Amersham (England). H^+ ion exchange resin Lewatit SPC 118 BG from Bayer was kindly provided by the Chemischefabrik, Brugg, and regenerated by two successive treatments with 2N NaOH and 2N HCl, then extensively washed with doubly distilled water.

Standard conditions for HMF synthesis and purification :

In a 10-20ml round bottomed flask equipped with a small magnetic bar and an

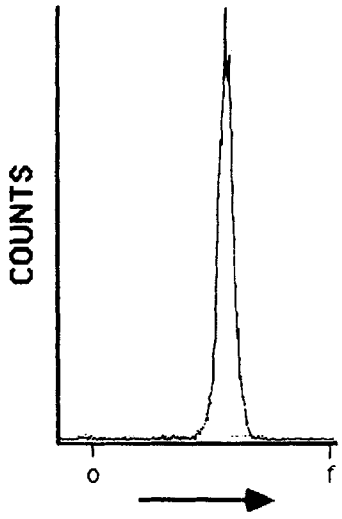


FIGURE 2 : TLC radiochromatogram of HMF after HPLC purification (o: origin, f: front)

all-glass stopper, D-[U-^{14}C]fructose (200µCi, 130µg) and cold D-fructose (145µg) were dryed under vacuum, then dissolved in 50 µl H_2O. A 10mg quantity of dry Lewatit SPC 118 BG ion exchange resin was regenerated and while still wet was added to the above suspension along with 450µl of isobutylmethylketone (IMK). The reaction was stirred and maintained at 88°C for 45min in an oil bath. At the end of the reaction, the flask was cooled in an ice bath, the IMK was recovered by pipetting and the resin washed first with 1ml IMK and then with 1ml water. The water phase was then extracted twice with two volumes of IMK. The IMK phases were pooled, the reaction products dried under vacuum and dissolved in methanol-H_2O (2:8,v/v). The [U-^{14}C]HMF was purified by injection in two parts on a Varian 5000 liquid chromatograph equipped with a reverse phase column (Valco LC-18, 5µ, 25cm, ID 4.6mm) using methanol-H_2O (2:8,v/v) as eluent at a flow rate of 1.2ml/min. HMF elution was monitored by UV detection at 283 nm.

TLC analysis:

The chemical purity and the reaction progress were monitored by thin layer chromatography on TLC plates coated with silica gel (Kiselgel GF 254, Merk)

eluted with ethanol. Reaction products were visualized either under UV light at 254nm or by radioscanning using a Berthold automatic TLC linear analyser (LB 2832). The radioactivity peaks were integrated with a Le Croy 3500 computer.

Acknowledgements

We thank R. Fumeaux, G. Philippossian and I. Horman for helpful discussions and critical reading of the manuscript and C. Isom for typing it.

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