

A CONVENIENT SYNTHESIS OF CARBOXYL-LABELLED RICINOLEIC ACID

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SUMMARY

The exchange reaction of carboxyl groups with labelled carbon dioxide has been utilized for the preparation of carboxyl labelled ricinoleic acid. The thermal instability of ricinoleic acid is overcome by pyrolysis of the t-butyl ether-t-butyl ester in the presence of labelled carbonate. The developed procedure should be appropriate to other thermally unstable substrates.

Key Words: Ricinoleic acid, ^{14}C exchange, Thermally unstable substrates

INTRODUCTION

Szammer and coworkers,¹ as a result of the study of the mechanism of ketone formation during pyrolysis of mixtures of carboxylic acid salts, have developed a convenient method for the formation of carboxyl labelled aliphatic acids. Important advantages of the method are that simple apparatus is required, the conversions require short times, the starting materials are labelled carbon dioxide and the non-labelled carboxylic acid, and the labelled residual carbon dioxide may be easily recovered from the reaction mixture.

A serious disadvantage of the method is the requirement for thermal stability of the carboxylic acid to be labelled.

A need for labelled ricinoleic acid of natural configuration has prompted us to investigate its synthetic preparation. Attempts to prepare the material by biosynthesis² proved unsatisfactory due to low yields of acid and poor

incorporation of labelled precursor leading to unsatisfactorily low specific activity ricinoleic acid. Initial attempts to prepare carboxyl labelled ricinoleic acid by the Szammer method were likewise unsuccessful due to the thermal instability of the starting acid.³ This instability of ricinoleic acid is due to the β -hydroxy olefinic moiety present in the substance. Masking this functionality by formation of an ether (rather than an ester which would likewise be thermally unstable) seemed to offer a possible solution. An ether should be thermally stable and stable to the basic carboxylate salt under the pyrolysis conditions. The *t*-butyl ether was chosen because of its ease of formation and cleavage⁴ under conditions which should not racemize the center of asymmetry in the starting ricinoleic acid. It was expected that the *t*-butyl ester should also be formed at the same time as the ether but that the ester should cleave under the pyrolysis conditions yielding the acid which should react to lead to labelled product.

RESULTS AND DISCUSSION

Commercial, practical grade sodium salt (Matheson, Coleman, Bell, Division of Matheson Co. Inc., East Rutherford, N. J., lot #14) of ricinoleic acid was purified as follows. The acid was recovered by ethereal extraction of an acidified aqueous solution of the sodium salt. The free acid was transformed to its lithium salt with lithium carbonate in aqueous methanol, the salt dried and digested 5 times with anhydrous ether. The material became increasingly easier to filter after each digestion with ether. The recovered, air dried lithium salt was reconverted to the free acid which still showed the presence of some slight impurities by TLC analysis (silica gel; chloroform, ethyl acetate, glacial acetic acid: 40:20:1-pre equilibrated plate). The acid was finally purified by chromatography over silica gel (elution with 25% ether in petroleum ether (60-65^o) containing 1% glacial acetic acid). In this manner material homogeneous by TLC and with suitable physical properties⁵ was obtained in about 50% yield from the practical salt.

The *t*-butyl ether - *t*-butyl ester was formed using the method of Beyenman and Heiszwolf,⁴ and purified by chromatography over silica gel (elution with 5% ether in petroleum ether).

The thermal stability of the ricinoleic acid derivative to the reaction conditions was examined by subjecting the t-butyl ester-t-butyl ether to pyrolysis at 320°. Recovered (70-85%) ricinoleic acid t-butyl ether was cleaved with trifluoroacetic acid and the free hydroxy acid purified. The recovered material showed physical, spectral and optical properties identical with authentic, natural ricinoleic acid.

The labelling procedure was tested using both ^{13}C labelled and ^{14}C labelled carbonate. Ricinoleic acid t-butyl ether-t-butyl ester was transferred to a reaction tube containing labelled carbonate (potassium carbonate, 50 atom % enriched, for ^{13}C or a mixture of unlabelled potassium carbonate and labelled sodium bicarbonate ^{14}C , 59.4 mCi/mmol), the tube was sealed under vacuum and heated for 140 minutes at 310-320° in a fluidized sand bath, while rotated. The tube was opened and the material recovered by dissolution in organic solvent. A pmr analysis in the case of the ^{13}C enriched reaction showed the t-butyl ester to be completely absent and the t-butyl ether to be essentially intact. The t-butyl ether was cleaved with trifluoroacetic acid⁴ and the labelled ricinoleic acid purified by preparative thin layer chromatography (silica gel; chloroform, ethyl acetate, glacial acetic acid, 80:20:1).

For the carbon-13 case pure ricinoleic acid was recovered in 58% yield with an enrichment of 8.3%. The enrichment was determined by mass spectral analysis of the trimethylsilyl ether-trimethyl silyl ester. In the case of the ^{14}C labelled acid the yield of recovered ricinoleic acid was 22% and represented a 13.5% radiochemical yield. Larger scale experiments using labelled samples have shown that the reaction is better yielding (70-85% of recovered ricinoleic acid) so the low yields in the case of the ^{14}C labelling experiment represent more a limitation of experimental expertise in handling the small quantities involved rather than of the procedure.

In summary we feel we have demonstrated that formation of the thermally and chemically stable t-butyl ether derivative of labile substances allows the extension of the direct carboxyl exchange reaction for isotopic labelling to a

class of substances otherwise very difficult to prepare. The concomitant formation of the t-butyl ester poses no problem since the free acid is regenerated directly under the reaction conditions by pyrolysis, reacts to form the salt and undergoes exchange with the CO_2 . The presence of a large surface area (glass beads or analogous materials) greatly enhances the rate of equilibration of label between the carboxylate salt and the CO_2 .

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