

PREPARATION OF [1-<sup>14</sup>C]- AND [9,10-<sup>3</sup>H]-*TRANS*-9-OCTADECENOIC ACIDS FROM THE CORRESPONDING *CIS*-COMPOUNDS.

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SUMMARY

[1-<sup>14</sup>C]-*Trans*-9-octadecenoic acid was obtained in 60 per cent overall yield and [9,10-<sup>3</sup>H]-*trans*-9-octadecenoic acid in 30 per cent yield by stereomutation of the double bonds in the corresponding *cis*-compounds with nitrous acid in toluene. The products were purified by silver nitrate chromatography and contained no detectable positional isomers.

Key words: *trans*-9-octadecenoic acid, double bonds - stereomutation.

INTRODUCTION

There is currently a great deal of interest in the biological properties of fatty acids containing *trans*-double bonds<sup>1</sup>. A variety of reagents has been used to effect the conversion of *cis*-double bonds of fatty acids to the *trans*-configuration<sup>2</sup> including selenium<sup>3,4</sup>, diphenylsulphide with UV irradiation<sup>4,5</sup> and nitrous acid<sup>2,6,7</sup>. All give an equilibrium mixture in which the thermodynamically more stable *trans*-isomer is present in the highest proportion (70 to 80 per cent) although the vigorous conditions required for isomerisation with some reagents can lead to considerable migration of the double bonds<sup>3</sup>. Nitrous acid, a reagent which is very easily prepared, appears to have been used hitherto only for the preparation of bulk quantities of fatty acids enriched in *trans*-isomers<sup>2,6,7</sup>. In this study, it was shown that it could be

adapted to the semimicro preparation of pure isotopically-labelled *trans*-monoenoic acids from the corresponding *cis*-compounds which were readily available commercially.

### RESULTS AND DISCUSSION

Nitrous acid, generated by addition of nitric acid to sodium nitrite, was found to effect rapid stereomutation of *cis*-double bonds in monoenoic fatty acids to the point when the equilibrium concentration of the *trans*-isomer was reached. Toluene was found to be a suitable solvent for the fatty acid during the reaction but somewhat erratic results were obtained with diethylether as solvent. Some polar byproduct formation always occurred but this could be controlled by using the minimum amount of dilute acid. For example, under the conditions described in the experimental section in five experiments, a mean of 68 per cent of the recovered radioactivity, added initially in the form of [1-<sup>14</sup>C]-*cis*-9-octadecenoic acid, was found in the *trans*-product, 23 per cent as the *cis*-isomer and 9 per cent as polar byproducts. When stronger acid (>2N) was used or the reaction time prolonged, the ratio of *trans*- to *cis*-products remained the same although the amount of each decreased while the proportion of polar byproducts increased to 30 per cent or more of the total.

Chromatography (gas-liquid) of the fragments obtained by von Rudloff oxidation<sup>8</sup> of the *trans*-product and of the *cis*-starting material gave a C<sub>9</sub> dibasic acid as essentially the only dibasic acid component (>99 per cent) from each so that no migration of the double bond had occurred during stereomutation. As no radioactivity was detectable in the oxidation products of the *trans*-component prepared from [9,10-<sup>3</sup>H]-*cis*-9-octadecenoic acid, no migration of the tritium atoms had occurred. Considerable double bond migration is known to occur when selenium is utilised to catalyse the reaction<sup>3</sup>.

The yield of tritium when [9,10-<sup>3</sup>H]-oleic acid was the starting material was consistently half of that anticipated on the basis of recoveries obtained with [<sup>14</sup>C]-labelled fatty acids in the same circumstances. Such a result was not expected on the basis of a proposed mechanism for the reaction<sup>2</sup> i.e. free radical addition of nitrogen dioxide at the double bond permitting free rotation of the carbon-carbon bond followed by removal of the radical. In an experiment in which the starting *cis*-compound was dually labelled (<sup>3</sup>H:<sup>14</sup>C = 1.156), 60 per cent of the added <sup>14</sup>C but only 27.8 per cent of the <sup>3</sup>H (<sup>3</sup>H:<sup>14</sup>C = 0.534) was in the *trans*-product while 19.9 per cent of the <sup>14</sup>C and 4.4 per cent of the <sup>3</sup>H were in the *cis*-product (<sup>3</sup>H:<sup>14</sup>C = 0.256) isolated from the reaction medium. The ratio of <sup>3</sup>H to <sup>14</sup>C (1.154) in the polar byproducts was virtually identical to that of the starting material. As monoenoic fatty acids labelled with tritium at the double bond are comparatively easily prepared with a high specific activity, such losses during stereomutation need not be considered unacceptable.

Nitrous acid and toluene then was shown to be a convenient mild reagent for stereomutation of isotopically-labelled fatty acids with *cis*-double bonds to the corresponding *trans*-isomers.

## EXPERIMENTAL

### Materials

[1-<sup>14</sup>C]- and [9,10-<sup>3</sup>H]-*cis*-9-octadecenoic acids (oleic acid) were purchased from the Radiochemical Centre, Amersham, England. The radioactivity of the starting materials and of any products of the reaction was checked by liquid scintillation counting of aliquots.

### Stereomutation of double bonds

The following procedure was found to give the most satisfactory results.

The *cis*-monoenoic fatty acid (3 to 10 mg; 50 to 5,000  $\mu$ Ci) was dissolved

in toluene (1 ml) and sodium nitrite (2 mg) and nitric acid (2N; 10  $\mu$ l) were added. The mixture was left in an atmosphere of nitrogen at room temperature for 1 hour, then water (2 ml) and hexane (10 ml) were added. The hexane layer was recovered and the solvent removed immediately by means of a rotary evaporator to eliminate the last traces of catalyst before the product was purified.

#### Thin layer chromatography on silver nitrate-impregnated layers

The procedure for separating *trans*-isomers described earlier was followed<sup>9</sup> except that 1 per cent formic acid was added to the developing solvent to effect separation of the free acids.

Yields of 60-65 per cent of the starting materials by weight were obtained in the form of the purified *trans*-compounds. A similar yield of radioactivity was obtained from the [1-<sup>14</sup>C]-acid but approximately half of this from the [9,10-<sup>3</sup>H]-acid.

#### Permanganate-periodate oxidation

In order to confirm the position of the double bond, unsaturated acids were cleaved by permanganate-periodate oxidation using the procedure of von Rudloff<sup>8</sup>. Further experimental details were given earlier<sup>9</sup>.

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