PREPARATION OF ERUCIC AND NERVONIC ACIDS LABELLED WITH CARBON-14[1]

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ABSTRACT

The malonic ester synthesis of nervonic acid (tetracos-15-enoic acid) has been modified so that the product consists of pure *cis* rather than a mixture of *cis* and *trans* isomers. The modified synthesis has been used for the preparation of C^{14}-labelled erucic and nervonic acids.

INTRODUCTION

The work reported in this paper was undertaken as a result of earlier experiments in this laboratory which indicated that erucic acid in some way influenced cholesterol metabolism in the rat. The addition of this monounsaturated C_{22} fatty acid to the diet caused an increase in the cholesterol content of the adrenals and liver and an increased excretion of cholesterol in the feces (3). Similar but less marked changes were also obtained by feeding the corresponding C_{20} (eicosenoic) and C_{24} (nervonic) acids. Erucic and eicosenoic are major component fatty acids of rapeseed oil (4), which is used in European countries as a salad oil and for incorporation into margarine. Nervonic acid occurs in the cerebrosides and sphingomyelin of the central nervous system, and is identical with selacholeic acid isolated from shark liver oil (7).

$CH_3—(CH_2)_7—CH=CH—(CH_2)_7—COOH$	Oleic acid
$CH_3—(CH_2)_7—CH=CH—(CH_2)_9—COOH$	Eicosenoic acid
$CH_3—(CH_2)_7—CH=CH—(CH_2)_{11}—COOH$	Erucic acid
$CH_3—(CH_2)_7—CH=CH—(CH_2)_{13}—COOH$	Nervonic acid

In studying the mechanism of this effect on cholesterol metabolism, it seemed desirable to have a method for incorporating a C^{14} label into these fatty acids. A synthesis of nervonic acid has been described (6, 10) in which erucic acid is reduced to the corresponding alcohol, which is then converted to the bromide and thence to nervonic acid by a malonic ester condensation. This method was used to obtain nervonic acid for our earlier feeding experiments (2), but it is unsatisfactory, particularly from the point of view of incorporating a radiocarbon label, because the final product consists of a mixture of *cis* and *trans* isomers from which it is difficult to obtain a high yield of the desired *cis* isomer.

An investigation of the various reactions of this synthetic route disclosed that the isomerization of the double bond occurred during conversion of the alcohol to the bromide, and it seems likely that hydrogen bromide liberated during the reaction was responsible. By omitting the heating step in this reaction it was possible to obtain nearly pure *cis* nervonic acid in approximately the same yield as the mixture of isomers obtained by earlier workers. The modified method has been used to prepare eicosenoic, erucic, and nervonic acids starting from oleic, eicosenoic, and erucic acids respectively, and the C^{14} label was introduced by using radioactive malonic ester for the condensation.

Although this synthesis proved satisfactory for the preparation of labelled fatty acids, the relatively low yield encountered in converting the fatty alcohols to the corresponding

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bromides was still a disadvantage in preparing large amounts of unlabelled fatty acids for feeding experiments. An alternative method has been investigated for the preparation of nervonic acid in which erucyl methanesulphonate was used instead of erucyl bromide as the intermediate. This doubled the over-all yield from erucyl alcohol to nervonic acid and resulted in a product which contained no *trans* isomer.

The fact that chain extension by means of a malonic ester condensation can now be achieved without isomerization of the double bond provides further confirmation of the stereochemical configuration of the higher homologues of oleic acid (1, 9).

EXPERIMENTAL

Fatty Alcohols

Erucyl alcohol was prepared by adding 100 g. (0.285 mole) of methyl erucate slowly to a solution of 6.7 g. (0.177 mole) of lithium aluminum hydride in 700 cc. of anhydrous ether in a three-necked flask equipped with stirrer and condenser. The reaction mixture was poured into ice water and acidified with 300 cc. of 3 *N* sulphuric acid. The ether layer was separated, washed with water, dried over sodium sulphate, and the product distilled. Yield—80 g. (87% of theory), $b.p._{0.5}$, 188–190° C. Oleyl alcohol and eicosenoyl alcohol were prepared in similar fashion. The methyl eicosenoate and methyl erucate used for these experiments were highly purified preparations provided by Dr. B. M. Craig of the Prairie Regional Laboratory, Saskatoon. Methyl oleate was prepared from oleic acid (Eastman Kodak) and purified by fractional distillation (2).

As an alternative to synthesis, crude oleyl and erucyl alcohols may be obtained from the Archer-Daniels-Midland Company, Midland, Michigan, under the names of ADOL 85 and ADOL 22 respectively. Erucyl alcohol from this source was purified by fractional distillation and used for our large-scale preparations of nervonic acid.

Alkyl Bromides

The method used for converting fatty alcohols to the corresponding bromides is illustrated by the following preparation of erucyl bromide. A solution of 195 g. (0.60 mole) of erucyl alcohol in 600 cc. of toluene was cooled in an ice bath and 60 g. (0.22 mole) of phosphorus tribromide in 300 cc. of toluene was added over a period of 1½ hours with stirring. The temperature of the reaction mixture was maintained between 0° and 5° C. during the addition of the tribromide and the product was worked up immediately without further heating. (In several runs the reaction mixture was allowed to stand for 1–4 hours at room temperature but some isomerization of the double bond occurred even under these conditions.) The toluene was removed *in vacuo* at 40–50° C. and the residue was diluted with 750 cc. of ether and washed first with two 150-cc. portions of a solution containing 10% potassium hydroxide and 10% sodium chloride and then with two 150-cc. portions of 10% sodium chloride solution. Emulsification tends to occur during the washing so the bottom layer was centrifuged after each washing and the upper phase returned to the separatory funnel. It was also found essential to dry the ether solution with magnesium sulphate rather than sodium sulphate; otherwise the mixture foamed badly during the subsequent distillation. Typically, the erucyl bromide distilled at 205–210° C. at 0.6 mm. and towards the end of the distillation the vapor temperature dropped somewhat and a more oily liquid began to distill. This was accompanied by a highly volatile gas which was not condensed in a dry-ice trap and which tended to ignite on contact with air. It is therefore best to discontinue the distillation when the temperature begins to drop, particularly since further distillation does not

appear to increase the subsequent yield of nervonic acid. The erucyl bromide weighed 105 g. (45% of theory).

Malonic Ester Condensation

To a solution of 5.8 g. (0.25 mole) of sodium in 150 cc. of absolute alcohol was added 45.5 g. (0.285 mole) of diethyl malonate. The solution was heated to boiling, then 96 g. (0.25 mole) of erucyl bromide was run in slowly and the mixture was refluxed for 1½ hours. Most of the alcohol was then distilled off and 550 cc. of water containing 5.5 cc. of concentrated hydrochloric acid was added to the residue. The product appeared as an oily top layer which was separated from the aqueous layer and hydrolyzed by refluxing with 160 g. of potassium hydroxide in 1 liter of 60% ethanol for 1½ hours. Part of the alcohol was removed by distillation, then 500 cc. of water was added and the solution was extracted with three 500-cc. portions of ether to remove neutral unreacted material. (This step was included in cases where it was desired to examine the nature of the substituted malonic acids and as a precaution in the preparation of radioactive fatty acids, but ordinarily it can be omitted (6).) The aqueous layer was then acidified with 180 g. of concentrated sulphuric acid in 1500 cc. of water and re-extracted with ether to obtain the crude erucyl malonic acid.

When the original method of Hale, Lycan, and Adams (6) was used for preparing erucyl bromide, the erucyl malonic acid obtained from it consisted of a mixture which was separated by fractional crystallization from chloroform – petroleum ether into two isomers melting at 101–102° C.* and 76.5–79° C. respectively. Calc. for $C_{25}H_{46}O_4$: C, 73.1; H, 11.3; neutral equivalent 205. Found,† high melting isomer: C, 72.5; H, 11.2; neutral equivalent 202; low melting isomer: C, 72.6; H, 11.0; neutral equivalent 204. The high melting isomer gave *trans* nervonic acid when decarboxylated by heating at 175° C. for 30 minutes. The low melting isomer gave *cis* nervonic acid on decarboxylation and was converted to the high melting isomer by treatment with dilute nitric acid and sodium nitrite (6). When the modified method for preparing erucyl bromide was used, the erucyl malonic acid consisted almost entirely of the low melting isomer (m.p. 77.5–78.5° C. after a single recrystallization from petroleum ether (35–50° C.) containing 10% chloroform).

The substituted malonic acids obtained from oleyl bromide and eicosenoyl bromide melted at 65–66° C. and 72–73° C. respectively. Calc. for $C_{21}H_{38}O_4$: C, 71.1; H, 10.8. Found: C, 70.9; H, 10.3. Calc. for $C_{23}H_{42}O_4$: C, 72.2; H, 11.1. Found: C, 72.3; H, 10.8.

Eicosenoic, erucic, and nervonic acids obtained by the modified synthetic method melted at 21–22° C., 31.5–32° C., and 38–39° C. after distillation and recrystallization from methanol. These figures are in general agreement with published data (1, 6, 8, 10). The eicosenoic acid prepared by Fieser and Chamberlain (5) appears to have been mainly the *trans* isomer.

The synthesis of radioactive fatty acids was carried out by essentially the same method as that used for the larger scale preparations. Diethyl malonate-1,3-C^{14} (145 mg. = 1 millicurie) from the Radiochemical Centre, Amersham, was diluted with 18 volumes of non-labelled diethyl malonate and the condensations were carried out on a 1.2 millimole scale. The synthetic fatty acids had specific activities of 6 to 8×10^4 counts/min./mg. (measured in a gas-flow counter—Nuclear, Chicago) after purification by distillation. In

**All melting points are corrected.*

†Microanalyses were performed by the Schwartzkopf Microanalytical Laboratory, 56–19 37th Avenue, Woodside 77, N.Y.

these syntheses, approximately half of the radioactivity was lost during decarboxylation of the substituted malonic acids, but this loss could be avoided by using ethyl malonate-2-C^{14}, in which case the fatty acids would be labelled in carbon-2 rather than in the carboxyl carbon.

Erucyl Methanesulphonate

A solution of 48.5 g. (0.150 mole) of erucyl alcohol in 150 cc. of pyridine (dried by distilling from barium oxide and storing over solid potassium hydroxide) was cooled in an ice bath and 18.0 g. (0.157 mole) of methanesulphonyl chloride (Eastman Kodak) was added. The mixture was stirred for 1 hour in the cold and then for 3 hours with the ice bath removed. The reaction mixture was poured into ice water and extracted with ether. The ether extract was washed with dilute hydrochloric acid to remove pyridine, then with water until neutral, and dried with sodium sulphate. Removal of the ether gave a product (54.5 g., 85% of theory) which melted at 33° C. after recrystallization from ethanol. Calc. for $C_{23}H_{46}O_3S$: C, 68.6; H, 11.5. Found: C, 68.5; H, 11.7.

For condensation with ethyl malonate, the crude methanesulphonate was recrystallized from 3 volumes of methanol at 0° C., and the reaction was carried out in a manner similar to that used for erucyl bromide. From 34 g. (0.082 mole) of erucyl methanesulphonate, there was obtained 22 g. (74% of theory) of distilled nervonic acid, b.p. 225–235° C. at 0.1 mm., m.p. 38–39.5° C.

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