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NOTES

Synthesis of 1,2-Dioleoyl-3-(α-^{14}C-1-adamantoyl)-*sn*-glycerol and 1-^{14}C-Adamantanecarboxylic Acid

ANTHONY J. VILLANI [x] and FRANCIS R. PFEIFFER

Abstract □ The pancreatic lipase inhibitor 1,2-dioleoyl-3-(α-^{14}C-1-adamantoyl)-*sn*-glycerol, with a specific activity of 8 mCi/mmole, was prepared by consecutive acylation of 1,2-isopropylidene-*sn*-glycerol with 1-^{14}C-adamantanecarboxylic acid chloride and oleoyl chloride. The ^{14}C-labeled acid was conveniently prepared by carboxylation of 1-adamantanol using ^{14}C-sodium formate in concentrated sulfuric acid.

Keyphrases □ 1,2-Dioleoyl-3-(α-^{14}C-1-adamantoyl)-*sn*-glycerol—pancreatic lipase inhibitor, synthesized □ 1-^{14}C-Adamantanecarboxylic acid—synthesized by carboxylation of 1-adamantanol using ^{14}C-sodium formate in sulfuric acid □ Pancreatic lipase inhibitor—1,2-dioleoyl-3-(α-^{14}C-1-adamantoyl)-*sn*-glycerol synthesized □ Inhibitors, pancreatic lipase—1,2-dioleoyl-3-(α-^{14}C-1-adamantoyl)-*sn*-glycerol synthesized

The investigation of biological applications of novel synthetic lipids has led to the study of pancreatic lipase inhibitors (1). The triglyceride 1,2-dioleoyl-3-(α-^{14}C-1-adamantoyl)-*sn*-glycerol (I) was found to be an inhibitor of pancreatic lipase when tested *in vitro* with isolated enzyme.

The mode of inhibition may involve covalent bonding of the 1-adamantoyl group of I to the enzyme. If the acylated enzyme could be isolated, then the role of the sterically hindered adamantoyl group in the mechanism of inhibition might be delineated.

The carbonyl carbon of the adamantoyl moiety of I was labeled with carbon-14 to test this theory. The synthesis of I is shown in Scheme I. *In vivo* biological data of I in rats will be reported elsewhere.

DISCUSSION

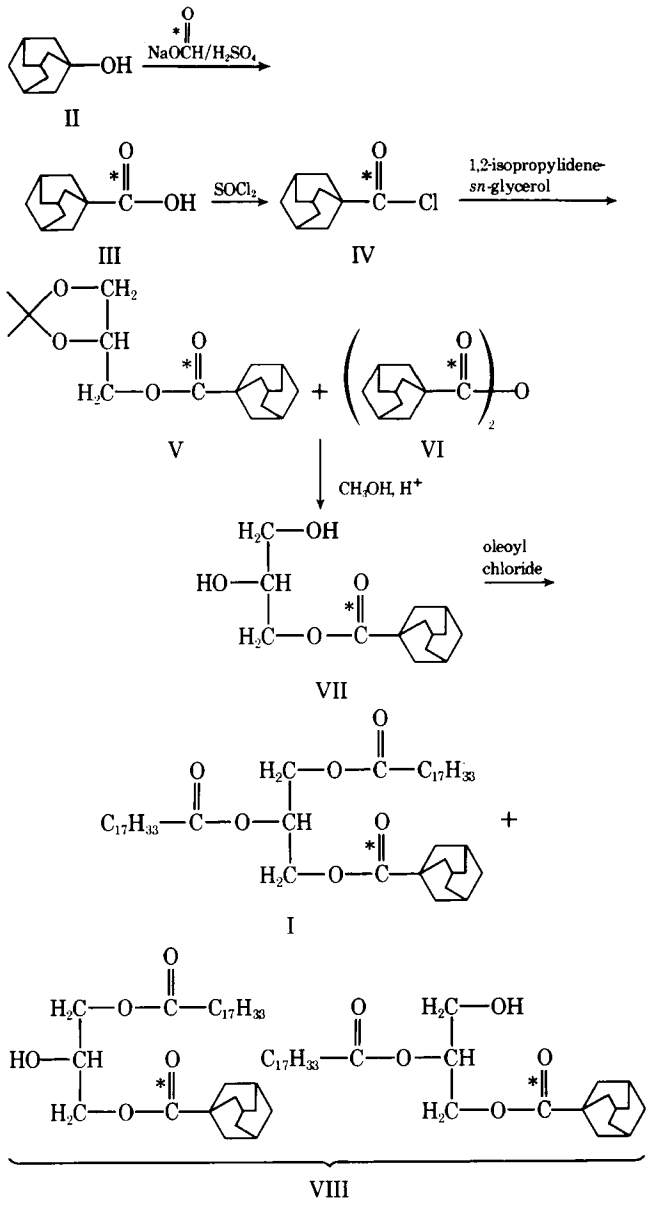
Since the literature procedure (2) for the carboxylation of 1-adamantanol was not readily adaptable to the semimicro radiosynthetic preparation, a modification was developed. In the present procedure, ^{14}C-carbon monoxide was generated *in situ* from ^{14}C-sodium formate and concentrated sulfuric acid. When this reaction was run in the presence of the adamantane cation, generated *in situ* from 1-adamantanol (II), the desired carboxylation was achieved and adamantanecarboxylic acid (III) was obtained in 82% yield. This procedure and the alternative method of Majerski *et al.* (3) provide convenient routes for the introduction of a carbon label in adamantane.

The carboxylic acid III was readily converted (4) to the acid chloride (IV) with thionyl chloride in refluxing benzene. The esterification of IV with 1,2-isopropylidene-*sn*-glycerol (5, 6) in the presence of pyridine was attended by the formation of the anhydride of adamantanecarboxylic acid (VI). The ratio of the ester V to the anhydride VI was identified (Fig. 1) as approximately 2:1 after 20 hr of refluxing in methylene chloride. The maximum yield of V could be obtained by the addition of excess isopropylideneglycerol and an increased reaction time.

Compound VI was also isolated and identified in cold runs and compared to an authentic sample of adamantanecarboxylic anhydride prepared by the procedure of Stetter and Rauscher (7).

The isopropylidene protective group was cleaved by mild acid to give the diol VII, which was used without purification. The acylation of VII with oleoyl chloride (8, 9) in the presence of pyridine gave crude I as a clear oil. After purification by magnesium silicate[1] column

[1] Florisil.



Scheme I

chromatography, I was isolated as an oil and had a radiopurity of 94.3% (Fig. 2) and a specific activity of 8 mCi/mmole. The only radioactive impurity was the diglyceride VIII, which amounted to 5.5%.

The labeled triglyceride was stored at −10° without desiccant. Reassay after 4 years showed a drop in radiopurity to 70.4%. The poor shelflife of the triglyceride was attributed to the formation of di- and monoglycerides by slow hydrolysis (10) under storage conditions and not to self-radiolytic decomposition.

EXPERIMENTAL[2]

1-^{14}C-Adamantanecarboxylic Acid (III)—To a mixture of 1-adamantanol (1.06 g, 7 mmoles) and ^{14}C-sodium formate (0.4 g, 6 mmoles, 40 mCi) in a 25-ml flask, fitted with a magnetic stirring bar and cooled in an ice–water bath, was added concentrated sulfuric acid

[2] ^{14}C-Sodium formate (6.8 mCi/mmole), purchased from New England Nuclear Corp., Boston, Mass., had a radiopurity of 99%. The specific activities were determined with a Packard Tri-Carb scintillation spectrometer (model 3003), which had a carbon-14 counting efficiency of 80%. The radiopurity of the labeled compounds was determined by thin-layer scanning, using the Berthold radioscanner/integrater (model 6000-10). Methylene chloride solvent was distilled over phosphorus pentoxide, and pyridine base was distilled over barium oxide. A thin-layer system of 3:1 (v/v) cyclohexane–ethyl acetate on silica GF was used throughout except where noted. 1,2-Isopropylidene-*sn*-glycerol was 95% pure by vapor phase chromatography.

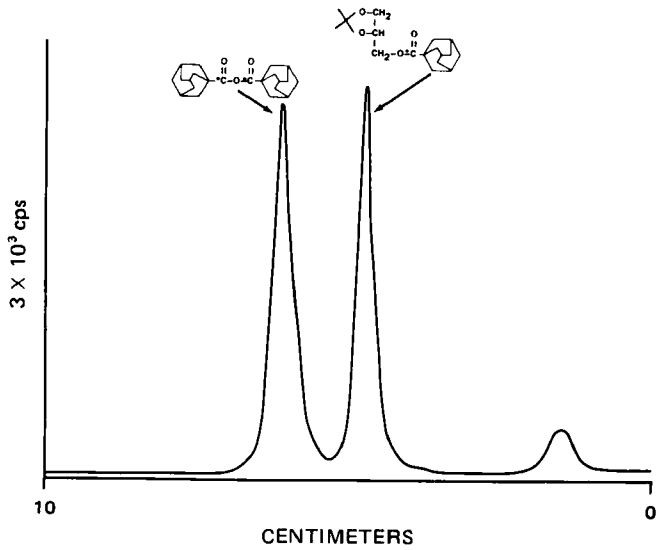


Figure 1—*Radiochromatogram of V (after 20 hr of refluxing in methylene chloride) using silica gel GF and a solvent system of cyclohexane–ethyl acetate (3:1 v/v).*

(40 ml) in one portion. The flask was quickly stoppered and allowed to reach room temperature gradually. The clear reaction mixture was then stirred at room temperature for 22 hr, warmed at 50° for 3 hr, and then poured over ice. The white solid precipitate was collected and dried under aspirator vacuum at 50°. The solid weighed 1.203 g (113% based on ^{14}C-sodium formate).

Purification was effected by dissolving the solid in freshly prepared 5% sodium bicarbonate solution, filtering, and acidifying with 3 *N* hydrochloric acid to pH 1. The solid was filtered and dried under aspirator vacuum at 50°. The product (0.866 g, 33 mCi, mp 170.5–175°) had a radiochromatographic purity of 98.8% as determined by TLC on silica gel GF, using a solvent system of ethyl acetate–acetic acid (99.5:0.5 v/v). The radiochemical yield was 82% (based on ^{14}C-sodium formate) with a specific activity of 7.2 mCi/mmole.

1-^{14}C-Adamantanecarboxylic Acid Chloride (IV)—A mixture of 1-^{14}C-adamantanecarboxylic acid (0.55 g, 3.1 mmoles, 22 mCi) and thionyl chloride (1 ml, 13.8 mmoles) was heated at reflux for 30 min. The reaction mixture was cooled and evaporated to dryness under aspirator vacuum. The residual white solid was used directly in the next step. Total chloride assays of 100% were routinely obtained in cold runs.

1,2-Isopropylidene-3-(α-^{14}C-1-adamantoyl)-*sn*-glycerol (V)—To a solution of 1,2-isopropylidene-*sn*-glycerol (0.79 g, 6 mmoles) and pyridine (0.475 g, 6 mmoles) in methylene chloride (6 ml) was

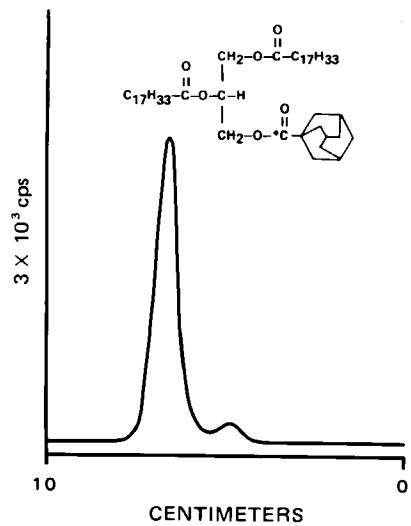


Figure 2—*Radiochromatogram of I using 0.4* N *boric acid–silica gel GF and a solvent system of chloroform–acetone–formic acid (96:4:0.4).*

added dropwise, at 0° and under nitrogen, a solution of IV in methylene chloride (5 ml). The addition took 10 min. The reaction was allowed to reach room temperature and then refluxed for 24 hr. At that time, a 30% excess of isopropylidene-*sn*-glycerol was added and the reaction was refluxed for an additional 16 hr or until TLC indicated maximum conversion.

The reaction mixture was diluted with ether and washed successively with 3 *N* hydrochloric acid, 5% sodium bicarbonate, and water. The bicarbonate extract was acidified with concentrated hydrochloric acid to give 1.92 mCi of 1-^{14}C-adamantanecarboxylic acid. The organic layer was dried over magnesium sulfate, filtered, and concentrated to dryness under aspirator vacuum. The residual oil was used directly in the next step. The radiochromatographic purity of the product was 95% as determined by TLC.

In a typical cold run, the reaction was stopped after 24 hr of refluxing in methylene chloride. After workup in the usual manner, the residual clear oil was stored in a stoppered glass vial at 4°. After a few days, a white solid crystallized out of the oil. The solid was triturated with cold acetonitrile and vacuum filtered to give 0.060 g, mp 204–205°; IR (KBr): 1810 and 1730 cm^{-1}; NMR ($CDCl_3$): 1.9 (m, 18H) and 1.7 (m, 12H) ppm; mass spectrum *m/e* 168, 135; identical to an authentic sample of adamantanecarboxylic anhydride prepared by the method of Stetter and Rauscher (7).

3-(α-^{14}C-1-Adamantoyl)-*sn*-glycerol (VII)—A solution of V in ether (2.5 ml), methanol (1 ml), and 3 *N* hydrochloric acid (0.4 ml) was stirred magnetically at room temperature for 16 hr. An additional 0.2 ml of 3 *N* hydrochloric acid was added, and the mixture was stirred for 15 hr or until TLC indicated complete conversion. The reaction mixture was diluted with ether and washed successively with water, 5% sodium bicarbonate, and saturated sodium chloride solution. The organic layer was dried over sodium sulfate, filtered, and concentrated to dryness under aspirator vacuum. The residual oil was used directly in the next step. The radiochromatographic purity of the product was 98.5% as determined by TLC.

1,2-Dioleoyl-3-(α-^{14}C-1-adamantoyl)-*sn*-glycerol (I)—To a solution of VII in methylene chloride (2 ml) was added pyridine (0.48 g, 6 mmoles) in methylene chloride (1 ml). The solution was cooled to 0–5° in an ice–water bath. To this magnetically stirred solution under nitrogen atmosphere was added dropwise oleoyl chloride (1.8 g, 6 mmoles) in methylene chloride (3 ml) over 5 min. The reaction was allowed to reach room temperature, stirred magnetically for 15 hr, diluted with ether, and washed successively with water, 3 *N* hydrochloric acid, 5% sodium bicarbonate, and saturated sodium chloride solution.

The ether solution was dried over sodium sulfate, filtered, and concentrated under aspirator vacuum to an oil. The oil was dissolved in 2 ml of petroleum ether–ether (1:1 v/v) and applied to a glass column packed with 50 g of magnesium silicate. The column was eluted with five 30-ml volumes of petroleum ether–ether (1:1 v/v). Fractions 4 and 5, which contained the desired product, were combined and evaporated to an oil under a vacuum of 5 μm. The residual oil weighed 1.35 g (13.8 mCi). The radiochemical yield was 63.8% (based on III), and the radiochromatographic purity was 94.3% as determined by TLC (Fig. 2); the specific activity was 8 mCi/mmole.

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Anticonvulsant Activity of Enzyme Inhibitors in Rats

K. RAMABADRAN *, M. BANSINATH, and M. N. GURUSWAMI ˣ

Abstract □ Three liver microsomal enzyme inhibitors, proadifen, 2,4-dichloro-6-phenylphenoxyethyldiethylamine, and 2,4-dichloro-6-phenylphenoxyethylamine, and a hepatotoxic agent, carbon tetrachloride, were tested for anticonvulsant activity in adult male albino rats using the maximal electroshock seizure technique. All four substances exhibited significant anticonvulsant activity 1 hr after intraperitoneal administration. This protection was absent when tested 24 hr later.

Keyphrases □ Enzyme inhibitors, liver microsomal—proadifen and two substituted ethylamines screened for anticonvulsant activity, rats □ Hepatotoxic agents—carbon tetrachloride, screened for anticonvulsant activity, rats □ Anticonvulsant activity—evaluation of liver microsomal enzyme inhibitors proadifen and substituted ethylamines, rats

Several reports indicated that enzyme inhibitors, when combined with antiepileptic drugs, increased the therapeutic effects of antiepileptic drugs (1–3). These results suggested the need to study the effect of enzyme inhibitors *per se* for anticonvulsant activity. Preliminary results are reported now.

EXPERIMENTAL

Adult male albino rats, 100–200 g, were allowed free access to food and water prior to testing. Anticonvulsant potency was determined by the maximal electroshock seizure test (150 mamp for 0.2 sec through ear clip electrodes) (4). Abolition of the hindlimb tonic extensor phase of the maximal electroshock seizure test was taken as the end-point for measuring anticonvulsant activity (5).

Five groups of animals (20–37/group) were used, one group each for proadifen[1] (β-diethylaminoethyl diphenylpropylacetate) (I), 2,4-dichloro-6-phenylphenoxyethyldiethylamine[2] (II), 2,4-di-

[1] Also designated by the code number SKF-525A; courtesy of Smith Kline and French Laboratories, Welwyn Garden City, Herts., England.

[2] Also designated by the code number Lilly 18947; courtesy of Eli Lilly.