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Biologically active lipids

Semi-synthesis of ^{3}H-labeled ether glycerophospholipids and ether glyceroglycolipids from ratfish liver oil

Helmut K. Mangold, Toshio Muramatsu°, Brian S. Roughley* and Nikolaus Weber

Bundesanstalt für Fettforschung, Institut für Biochemie und Technologie, H.P. Kaufmann-Institut, Piusallee 68, D-4400 Münster, FRG, °Tokyo Medical and Dental University, Department of General Education, Tokyo 272, Japan and **Amersham International plc, Cardiff Laboratories, Forest Farm Whitchurch, Cardiff CF4 7YT, Wales*

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1-*O*-Alkyl-2,3-diacyl-*sn*-glycerols, the major constituents of ratfish (*Chimaera monstrosa*) liver oil, serve as starting material for the preparation of 1-*O*-alkyl-*sn*-glycero-3-phosphocholines, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholines, 1-*O*-alkyl-2-*O*-methyl-*sn*-glycero-3-phosphocholines, and 1-*O*-alkyl-2-*O*-methyl-*sn*-glycero-3-β-D-glucopyranosides. Catalytic tritiation of the unsaturated alkyl moieties in these biologically active ether lipids affords the corresponding ^{3}H-labeled substances.

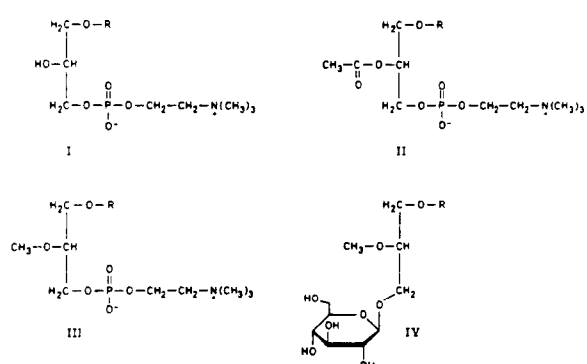
Ether lipid; Alkylglycerophosphocholine; Alkylacetylglycerophosphocholine; Alkylmethylglycerophosphocholine; Alkylmethylglyceroglucopyranoside; Platelet-activating factor

1. INTRODUCTION

Biologically active ether glycerophospholipids are of great current interest. 1-*O*-Alkyl-*sn*-glycero-3-phosphocholines (**I**) exhibit cancerostatic properties [1], 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholines (platelet activating factor, PAF) (**II**) are potent mediators of various physiological reactions [2], and 1-*O*-alkyl-2-*O*-methyl-*sn*-glycero-3-phosphocholines (**III**) show strong antineoplastic activities [1]. Ether glyceroglycolipids, such as 1-*O*-alkyl-2-*O*-methyl-*sn*-glycero-3-β-D-glucopyranosides (**IV**), may also be useful as cancerostatic agents [3].

The synthesis of these chiral compounds is rather tedious. In contrast, their semi-synthetic preparation from naturally occurring ether lipids can be carried out easily, even on a large scale.

The present communication describes the semi-synthesis of **I–IV** from the neutral ether lipids of ratfish (*Chimaera monstrosa*) liver oil, an abundant and inexpensive starting material.



Correspondence address: H.K. Mangold, Bundesanstalt für Fettforschung, Institut für Biochemie und Technologie, H.P. Kaufmann-Institut, Piusallee 68, D-4400 Münster, FRG

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Unsaturated alkyl moieties of these substances are catalytically tritiated. The resulting ^{3}H-labeled ether lipids are useful tools for biomedical and clinical research.

2. MATERIALS AND METHODS

Atlantic ratfish, *C. monstrosa*, that had been caught in the North Atlantic were supplied by the Federal Research Center for Fisheries, Hamburg, FRG. All reagents, adsorbents, and solvents were products of E. Merck, Darmstadt, FRG.

Melting points (m.p.) were determined on a Kofler hot stage under the microscope (Reichert, C. Wien, Austria) and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer spectral photometer 257 (Perkin-Elmer Bodenseewerk, Überlingen, FRG) in carbon tetrachloride solution. Optical rotations were measured on a Perkin-Elmer polarimeter 241 MC in chloroform or chloroform/methanol (1:1, v/v) solution. Gas chromatography was carried out on a Perkin-Elmer F22 instrument equipped with a flame ionization detector. 1-*O*-Alkyl-2,3-diacetyl-*sn*-glycerols were analyzed on a glass column, 1.8 m × 4 mm, packed with 10% Silar 5CP on Gas Chrom Q, 100–120 mesh (Applied Science Laboratories, State College, PA) at 250°C with nitrogen as carrier gas at a flow rate of 50 ml/min. The composition of alkyl chains (number of carbon atoms:number of double bonds) was found to be: 11% (16:0), 8% (16:1), 10% (18:0), 61% (18:1), 10% (others).

The course of reactions and the purity of final products were assessed by thin-layer chromatography on silica gel H with hexane/diethyl ether (7:3, v/v) as developing solvent for the analysis of the intermediate 1-*O*-alkyl-2-acyl-*sn*-glycerols and 1-*O*-alkyl-2-*O*-methyl-*sn*-glycerols, chloroform/methanol/water/acetic acid (70:35:5:5, by vol.) for the final products **I**, **II**, and **III** and chloroform/methanol (85:15, v/v) for **IV**. The various fractions were detected by charring after spraying the plates with chromic-sulfuric-acid solution.

The distribution of radioactive fractions on thin-layer chromatograms and the purity of **I**, **II**, **III** and **IV** were determined by scanning with a Berthold automatic TLC-linear analyzer LB 2832 in combination with a data acquisition system LB 500 (BF-Vertriebsgesellschaft, Wildbad, FRG).

All reactions were carried out in an atmosphere of pure nitrogen as far as possible. The water used for washing was made air-free by boiling and subsequent cooling in a stream of nitrogen.

1-*O*-Alkyl-2-acyl-*sn*-glycerols containing both saturated and monounsaturated alkyl moieties were obtained, on a 10 g scale, from the neutral ether lipids of ratfish liver oil by acid-catalyzed hydrolysis of the vinyl ether bond of the 1-*O*-(1-alkenyl)-2,3-diacyl-*sn*-glycerols followed by enzymatic cleavage of the ester linkages at positions 3 of the remaining 2,3-diacylglycerols and 1-*O*-alkyl-2,3-diacyl-*sn*-glycerols with pancreatic lipase [4]. The reaction mixture was resolved by chromatography on layers, 1 mm thick, of silica gel H with hexane/diethyl ether (2:3, v/v), the 1-*O*-alkyl-2-acyl-*sn*-glycerols were eluted from the adsorbent using diethyl ether saturated with water and phosphorylated immediately.

1-*O*-Alkyl-2-*O*-methyl-*sn*-glycerols containing both saturated and monounsaturated alkyl moieties were prepared, on a 10 g scale, from the 1-*O*-alkyl-*sn*-glycerols derived from ratfish liver oil by methanolysis or lithium aluminum hydride reduction followed by chromatography on a column (30 × 2.5 cm) of silica gel 60 using hexane/diethyl ether (95:5 to 10:90, v/v) as eluant. After protecting the primary hydroxy group of the 1-*O*-alkyl-*sn*-glycerols by tritylation [5], the secondary group was methylated with methyl iodide or methyl methanesulfonate [6]. Following removal of the trityl group with 10% aqueous HCl in methanol (1:5, v/v) [7], the reaction products were resolved by chromatography on layers of silica gel H, 1 mm thick, with hexane/diethyl ether (2:3, v/v), and the 1-*O*-alkyl-2-*O*-methyl-*sn*-glycerols were eluted from the adsorbent using diethyl ether saturated with water.

The 1-*O*-alkyl-2-acyl-*sn*-glycerols were used for the preparation of the phospholipids **I** and **II** [4], whereas the 1-*O*-alkyl-2-*O*-methyl-*sn*-glycerols were converted to the phospholipids **III** [4] and the glycolipids **IV** [6].

Aliquots of these ether lipids were radiolabeled with tritium by the reduction of the unsaturated alkyl moieties using a palladium catalyst and tritium gas. The tritiated compounds were purified by thin-layer chromatography on silica gel GF. Specific radioactivities were determined in conjunction with a phosphate assay [8].

3. RESULTS AND DISCUSSION

The 1-*O*-alkyl-*sn*-glycero-3-phosphocholines (**I**) and 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholines (**II**) were prepared in 29% and 24% yield, respectively, from the 1-*O*-alkyl-2-acyl-*sn*-glycerols derived from the neutral ether lipids of ratfish liver oil (yields are on the basis of crude oil); **I**: m.p. $>200°C$ (decomposition), $[\alpha]_D^{20} - 5.0°$ (chloroform/methanol, 1:1, v/v); **II**: m.p. $>200°C$ (decomposition), $[\alpha]_D^{27} - 2.8°$ (chloroform).

The 1-*O*-alkyl-2-*O*-methyl-*sn*-glycero-3-phosphocholines (**III**) were obtained in 81% yield by reacting 1-*O*-alkyl-2-*O*-methyl-*sn*-glycerols derived from ratfish liver oil with bromoethylphosphoric acid dichloride followed by trimethylamine; **III**: m.p. $>220°C$ (decomposition), $[\alpha]_D^{22} - 3.38°$ (chloroform).

The 1-*O*-alkyl-2-*O*-methyl-*sn*-glycero-3-β-D-(tetraacetyl)glucopyranosides were synthesized in 85% yield by the reaction of 1-*O*-alkyl-2-*O*-methyl-*sn*-glycerols with acetobromoglucose, m.p. $<30°C$, $[\alpha]_D^{20} - 11.1°$ (chloroform). Removal of the acetyl groups through alkaline hydrolysis yielded pure **IV**.

Thin-layer chromatograms and radio-scans thereof show that the chemical and radiochemical purity of the ^{3}H-labeled ether lipids described were better than 98%. Determinations of the products from several tritiations were in the range 60–110 Ci/mmol. Examination of typical batches of the tritiated ether lipids by ^{3}H-NMR indicated that the label was present in the alkyl chain exclusively. The tritiated substances were found to be subject to radiation-induced decomposition at a rate of 0.5 to 1%, per month. They must, therefore, be purified by chromatography at least twice a year.

^{3}H-labeled preparations of 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholines (**II**) derived from the neutral ether lipids of ratfish liver oil have been offered by Amersham International since 1982; they have found wide use in biomedical studies such as the determination of binding sites and the development of receptor antagonists of PAF [9]. The ^{3}H-labeled 1-*O*-alkyl-2-*O*-methyl-*sn*-glycero-3-phosphocholines (**III**) described are now also commercially available. This substance should be of value in studying the metabolism of such neoplastic agents [1] in cell cultures and whole animals.

The 1-*O*-alkyl-*sn*-glycerols and 1-*O*-alkyl-2-*O*-methyl-*sn*-glycerols used as intermediates also constitute valuable starting materials for the preparation of some PAF antagonists [9,10]. ^{3}H-labeled preparations could be used in studying the mode of action as well as the metabolism of such antagonists.

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