3-HYDROXY-5,8,11,14 (ALL CIS)-EICOSATETRAENOIC ACID (3-HETE) - A NEW ASPIRIN SENSITIVE ARACHIDONIC ACID METABOLITE FROM YEAST

M.S. VAN DYK1*, J.L.F. KOCK1, A. BOTHA1, D.J. COETZEE1, P.J BOTES1, O.P.H. AUGUSTYN2, S. NIGAM3

1Department of Microbiology and Biochemistry, University of the Orange Free State, PO Box 339, Bloemfontein, South Africa; 2Viticultural and Oenological Research Institute, Stellenbosch, South Africa; 3Eicosanoid Research, Department of Gynecology, University Medical Center Steglitz, Free University Berlin, D-1000 Berlin 45, F.R.G.

INTRODUCTION

Our group has recently reported evidence for the presence of prostaglandins in yeasts [1]. Radioimmunoassay of extracts of yeast belonging to the Lipomycetaceae and Saccharomyces cerevisiae (baker's yeast) revealed the presence of significant quantities of PGF2α. In a previous report we demonstrated that cultures of Dipodascopsis uninucleata produced significant amounts of an aspirin sensitive compound which showed distinct chromatographic properties to those of usual cyclooxygenase products [2].

The present paper reports chemical and spectroscopic data on this metabolite, the structure of which has been proposed as 3-hydroxy-5,8,11,14(all cis)-eicosatetraenoic acid (3-HETE).

RESULTS AND DISCUSSION

Cultivation and harvesting of yeast Dipodascopsis uninucleata were performed as described [1]. The extraction of arachidonic acid (AA) metabolites was done as described [2].

Autoradiography.

TLC-autoradiograms of ethanolic extracts from D. uninucleata cultures fed with tritium-labeled AA showed several aspirin-sensitive bands at Rf values unusual for prostaglandins (Figure 1, lanes 1 and 6). The concentrations of these metabolites could be increased by addition of increasing amounts of cold AA (Figure 1, lanes 2-5). Iodine stained TLC plates of purified extracts from yeast cells fed with AA showed the compound at Rf 0.13 as a major component [2]. It was isolated in pure form by scraping this band from the TLC plates and subjected to further investigations by gas chromatography-mass spectrometry (GC-MS), UV, IR and NMR spectroscopy.

Spectroscopic data on 3-HETE.

UV and IR spectra of this compound showed that it has no conjugated double bond or enone type system (UVmax at 193 nm), but a carbonyl absorption at 1710 cm⁻¹ and a broad absorption between 2800 cm⁻¹ and 3760 cm⁻¹. Accordingly, the compound is a hydroxy acid [data not shown].
Fast atom bombardment (FAB)-MS of the aspirin sensitive compound revealed a molecular weight of 320, suggesting a mono-hydroxy C20:4 fatty acid (data not shown). In order to determine the position of the hydroxyl group electron impact, (EI)-MS were obtained of methylated and methylated, hydrogenated fatty acid [2]. The spectra of these samples showed molecular ions of m/z 334 and 342, respectively, thus confirming the presence of 4 double bonds. Both samples gave base peaks of m/z 103 which indicates the fragment CH$_3$O(CO)CH$_2$CHOH and characterizes 3-hydroxy fatty acids [2]. EI-MS of methylated, trimethylsilylated probe also showed 3-HETE as a major aspirin inhibited metabolite of AA (Figure 2). The base peak of m/z 175 [CH$_3$O(CO).CH$_2$.CHO.TMSi] is characteristic for the C-3 hydroxylation of the fatty acid.

Comparison of the $^1$H-NMR spectrum (300 MHz, CDC$_3$) of the 3-hydroxy compound with that of AA showed that the 3-hydroxy compound has the same all cis double bond configuration as AA [2]. All signals in the $^1$H-NMR spectrum could be assigned after a 2D-COSY spectrum was obtained (Figure 3). Thus the compound could be described as 3-hydroxy-5,8,11,14(all cis)-eicosatetraenoic acid (3-HETE).

The aspirin inhibition of 3-HETE formation seems to be a strange process since 3-HETE is not a cyclooxygenase product. Preliminary studies on the reproduction of D. uninucleata demonstrated that the sexual phase of the life cycle was inhibited by aspirin [4]. The extent to which phospholipid metabolism in yeasts belonging to the genus Dipodascopsis mimics to that in mammalian cells is currently under investigation. For instance, it is well known that only yeasts of this genus produce ATP citrate lyase. We believe that these organisms might eventually serve as simple models for the mam-