Identification of Arachidonic Acid in Lipids of Petersia africana

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Summary: The lipid extract prepared from the bark of the West African Petersia africana was found to contain arachidonic compound (C.20:4) in substantial amount. The pure compound after isolation by preparative gas-liquid chromatography (glc) was identified by both physical and chemical methods. In addition other fatty acids linoleic (C 18:2), oleic (C 18:1), palmitoleic (C 16:1) myristoleic (C 14:1) lauroleic (C 12:1) were also found. The lipid fraction proved to contain triacylglycerol, cholesterol ester, hydrocarbons and glycosides. This is the first report of the detection of arachidonic compound in P.africana.

Introduction

P.africana, (Lecythidaceae) is a tall tree with bark deeply fissured, smelling strongly foetid when slashed [1]. This investigation was carried out in an effort to identify the active chemical principle which attracts the common Nigerian hawkmoth Deilephila neril (Sphingidae) preferentially to lay eggs in the bark of P.africana as a first step in its reproductive cycle. Extensive work on biochemicals known as pheromone attactants have been carried out for a few plants [2-5]. However due to the chemical diversity of the phernomone attactants already identified more effort was necessary to improve the understanding of the nature and role of such biochemicals.

Material and Method

Extraction of Lipids

Fresh bark of P.africana was macerated and homogenised in a domestic blender. 5g of the homogenised bark was extracted with chloroform-methanol (2:1 v/v) at room temperature. The extract was concentrated in vacuo, the water-soluble impurities in the concentrate were removed by washing the chloroform - methanol with a solution of 0.29% NaCl three times according to the method of Folch et al. [6].

Chromatography of Lipids

The investigation of the fatty acids and the lipids was carried out using a combination of thin-layer (tlc) and gas-liquid (glc) chromatographies. The fatty acid methyl esters were prepared by transesterification of the lipids according to Kates [7]. In this process an aliquot of the chloroform solution containing ca. 20 mg of the lipid was concentrated in a stream of dry nitrogen, and heated in an Erlenmeyer flask with side-arm 4.5 ml of methanol - HCl was added and refluxed for 90 min. over chips of dry calcium chloride. 0.5 ml of water

was added followed by methanol-water (9:1). The fatty acid methyl esters were extracted with pet.ether (3 x 10 ml) and dried over calcium chloride. The resulting solution was dried in a stream of dry nitrogen and applied directly on glc.

0.5 g of the chloroform extract of transmethylated hexane extract of the fatty acid was chromatographed isothermally on a 2.1 m x 6 mm (id) glass column packed with HI-EFF (Applied Science) on AW-DMCS Chromosorb W (80-100 mesh) using a Pye series 104 gas chromatography equipped with a flame ionisation detector (FID). Oxygen free nitrogen gas was used as the carrier gas at a flow rate of 50 ml/min. The fatty acids were identified through the use of authentic samples from Analabs inc. North-Haven Conn., and from the plot of the retention times against carbon number. Concentration of each component was calculated from peak areas. The glc conditions were optimised to obtain good resolution of the peaks. The peaks obtained approximated gaussian curves and the areas under the peaks were taken as heights of the peaks times the corresponding widths at half the peak heights. Under these conditions the volumes of the components are directly proportional to areas under the peaks.

The infrared spectrum was obtained with a Perkin-Elmer 257 grating infrared spectrophotometer.

Column chromatography using silicic acid (silica gel MFC 100-200 mesh Hopkins and Williams, Romford; England) was used to fractionate the total lipid extract into neutral and polar fractions. The adsorbent was prepared according to Rouser et al. [8] and elution was carried out by chloroform (200 ml) to yield the neutral fraction (1). This was followed by chloroform-methanol (200 ml.2:v v/v to give the polar fraction (fraction 2).

Lipid classes were identified through the use of authentic samples and by reference to relative R_f values and specific stain reagents.

Results

The glc of the fatty acids showed that oleic (C 18:1) acid 46% was the major component. This was followed by arachidonic (C 20:4) acid 17.5% and linoleic (C 18:2) acid 16.5%. The other fatty acids were myristoleic (C 14:1) acid 8%, palmitoleic (C 16:1), acid 6%. lauroleic (C 12:1) acid 3% and traces 3% of other lower fatty acids or degraded metabolites as unresolved peaks. There is no previous record of the lipids of this plant. By using preparative glc a pure sample of the arachidonic acid was obtained. The anlytical glc retention time was the same as an authentic sample from Analabs. Inc., North Haven, Conn., Mixed glc of the two sample gave a single symmetrical peak. Infrared of the arachidonic compound of P.africana showed bands at (1660, 1420, 1000, 730 cm^{-1} C-C and 1760, cm^{-1} 1230 cm^{-1} C-O). The iodine number determined according to standard method [9] showed the presence of four double bonds. Further evidence of structure was confirmed by U.V. according to Brice at al. [10]. Detailed structural investigations for the remaining fatty acids were not attempted. This was mainly because the occurence of these various fatty acids in plants is known.

Discussion

As far as the author is aware this is the first report of the presence of arachidonic acid in P.africana, and

this may be the reason for the strict host specificity of D.nerii on P.africana. D.nerii preferentially lays eggs in the bark of the plant where they hatch to large caterpillars. Not long ago these caterpillars were consumed as food by some tribe in Nigeria. Lately, scarcity of these caterpillars may be attributable to indiscriminate felling of the host plant. Certain metabolites called pheromone-attractants [2-4] exist in plants. It is not unlikely that arachidonic acid which is nutritionally essential in diet [11] and of great significance to D.nerii [12] may perform as pheromone, essential nutrient or act as a precursor to many other important metabolites through the known "arachidonic acid cascade system". However, because of the complexity of the mode of operation [13] of the chemical mediators much work still has to be done in order to establish the chemical basis of the role of this important acid to D.nerii. In view of this analysis of the lipids of this plant is being undertaken and will be reported later.

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