THE TRANSFORMATION OF PHYTOL INTO 3,7,11,15-TETRAMETHYL-HEXADECANOIC (PHYTANIC) ACID IN HEREDOPATHIA ATACTICA POLYNEURITIFORMIS (REFSUM'S SYNDROME)

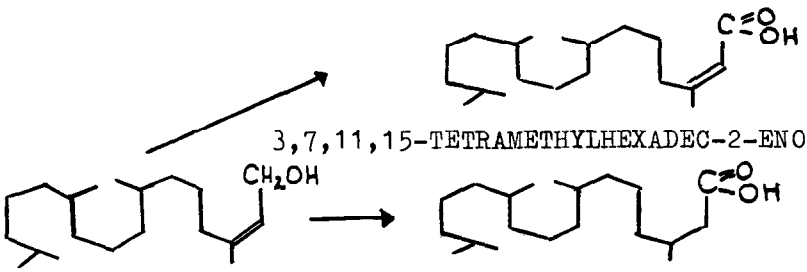
W. Stoffel and W. Kahlke

Physiologisch-Chemisches Institut, University of Cologne and Medizinische Kliniken, Heidelberg

Received January 26, 1965

Klenk and Kahlke (1963) discovered considerable amounts of 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid) in the lipids of liver, kidney, muscle, and urine of an infant, who died with heredopathia atactica polyneuritiformis. The total fatty acid mixture of the cholesterol ester and triglyceride fractions consisted of more than one half of this acid. It is also present in the serum lipids (Kahlke 1963). The occurrence of phytanic acid has been shown in nine patients with Refsum's syndrome and the distribution in different lipid fractions has been studied (Kahlke 1964).

The mode of the biosynthesis of this diterpenoic acid is unknown. One of several possible pathways is the transformation of an exogenous precursor, most likely phytol:



This possible pathway has been studied with tritium-labelled phytol.

[6,7,10,11,14,15-^{3}H] phytol has been synthesized starting from farnesol by the following reaction sequence: farnesol $\xrightarrow{PBr_3}$ farnesylbromide $\xrightarrow[\text{condensation}]{\text{acetoaceticester}}$ farnesylacetone $\xrightarrow{^3H_2/Pd}$ ^{3}H hexahydrofarnesylacetone $\xrightarrow{KC\equiv CH}$ [^{3}H] dehydroisophytol $\xrightarrow{H_2/\text{Lindlar catalyst}}$ [^{3}H] isophytol $\xrightarrow{PBr_3}$ [^{3}H] phytylbromide $\xrightarrow{KOOCCH_3}$ [^{3}H] phytylacetate $\xrightarrow{OH^-}$ [^{3}H] phytol $\xrightarrow{\text{phthalic anhydride}}$ [^{3}H] phytylphthalate $\xrightarrow{OH^-}$ [6,7,10,11,14,15-^{3}H] phytol.

This reaction sequence is essentially that of Ruzicka and Firmenich (1939) for the synthesis of geranylgeraniol, exept the catalytic hydrogenation step of farnesylacetone with tritium gas being introduced. The final purification was achieved by preparative TLC (hexane:ether:acetic acid 70:40:5, Rf 0,3-0,4). This chromatographically pure phytol had specific activity of 5 μC/μmole.

After a twelve hours fasting period, 72 mg (1,2 mC) phytol were fed to a male patient suffering heredopathia atactica polyneuritiformis (Harders and Dieckmann 1964). 20 cc samples of citrate blood were taken after 1,2,3,4,5,8, 12,24,30,36 and 48 hours, centrifuged, and the plasma lyophylized. The 1,5,12 and 24 hour samples were extracted three times with chloroform-methanol (2:1). The combined extracts of each sample washed with 0,04% $CaCl_2$-solution and concentrated under vacuum. The total lipid mixture of each sample was refluxed for 2 hours with 2 ml of a 2% (w/v) solution of hydrochloric acid in methanol. Following this incubation the methyl esters were extracted with light petroleum ether. The solvent was then evaporated and the residue saponified with 1N methanolic KOH. Unsaponifiable matter was carefully extracted with petroleum ether. After acidification, the free

fatty acids were extracted and again esterified with hydrochloric acid-methanol (5% w/v). Radiogaschromatography of the methyl ester mixture conclusively showed that the total radioactivity and methyl tetramethylhexadecanoate eluted together in one peak and co-chromatographed exactly with synthetic methyl tetramethylhexadecanoate. This is shown in Fig.1.

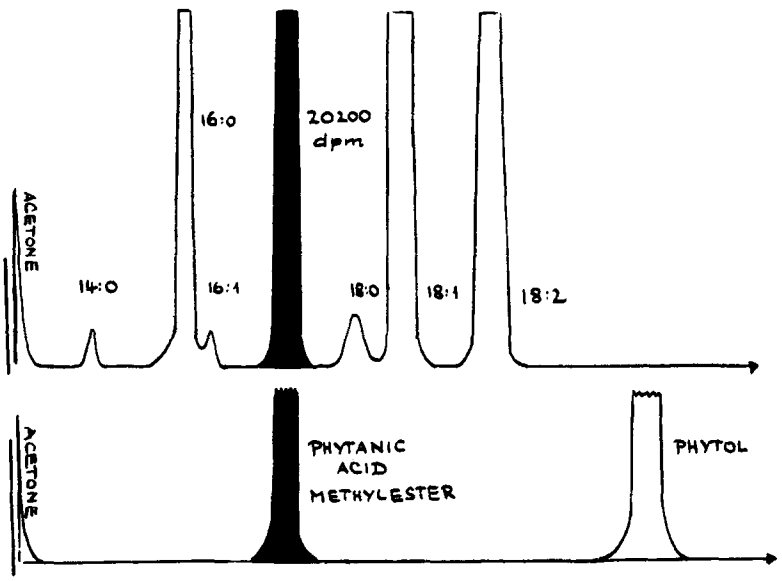


Fig.1 Radiogaschromatogram of the 5-hour-sample fatty acid methylester mixture
Column temperature 175°, EGS (15% on Chromosorb), 200 cm column length, 60 ml Ar/min., Barber Coleman 10.

The total radioactivity of the plasma fatty acids is identical with radioactive phytanic acid. The blood levels of four samples taken after different intervals and also calculated for the total blood volume (5 liters) are summarized in table 1.

Table 1

Plasma sample after	Radioactivity of 10 cc sample dpm	Radioactivity of total blood dpm	Radioactive phytanic acid µmoles
1 hour	20000	$2,0 \times 10^7$	1,9
5 hours	675000	$3,5 \times 10^8$	32,0
12 hours	310000	$1,55 \times 10^8$	14,0
24 hours	300000	$1,50 \times 10^8$	13,6

No α,ß unsaturated phytenic acid could be detected, because oxidative ozonolysis did not yield any tritium labelled hexahydrofarnesylacetone.

Little is known about the metabolism of phytol in man. No accumulation of phytanic acid has been observed in normal individuals. The rapid transformation of phytol into phytanic acid and the accumulation of this acid in lipids from patients with heredopathia atactica polyneuritiformis strongly suggest an metabolic error of tetramethylhexadecanoic acid rather than an intermediate of cholesterol synthesis as a precursor of this acid.

References

Harders,H.and Dieckmann,H. Dtsch.Med.Wschr. 89,248(1964)
Klenk,E.and Kahlke,W. Ztschr.f.Physiol.Chem. 333,133(1963)
Kahlke,W. Klin.Wschr. 41,783(1963)
Kahlke,W. Klin.Wschr. 42,1011(1964)