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Occurrence of Phytanic Acid in Rumen Bacteria

THE branched-chain fatty acid 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid) has been isolated and identified in butterfat[1–4], sheep fat[5], ox fat[6], ox serum[7,8], crude petroleum[9] and also in the tissues, serum and urine of humans afflicted with the rare disease referred to as Refsum's syndrome[10–12] (heredopathia atactica polyneuritiformis). This communication reports the occurrence of trace quantities of this acid in the rumen bacteria from a fistulated cow fed clover-grass hay.

Phytanic acid has not hitherto been reported as a constituent of rumen lipids, although Keeney, Katz and Allison[13], in their quantitative studies on rumen digesta, detected C_{13}, C_{14}, C_{15}, C_{16} and C_{17} branched-chain fatty acids in rumen bacteria. In the investigation now reported, a concentrate of phytanic acid was prepared from the rumen bacterial lipids by low-temperature crystallization from acetone and mercuric acetate adduct formation followed by chromatographic separation on a 'Florisil' column[14]. The presence of the C_{20} multi-branched component was determined by gas–liquid chromatography, recognition of the chart peak being based on retention data previously established in this laboratory for the same acid isolated from butterfat[4] and from Refsum's syndrome tissues[12] and conclusively identified by techniques which included mass and infra-red spectrometry. The methyl ester of authentic phytanic acid isolated from ox fat[6] when added to the methyl esters of the phytanic acid concentrate extracted from rumen bacteria, co-chromatographed on columns impregnated with polydiethylene glycol adipate and 'Apiezon L', respectively.

The presence of branched-chain fatty acids in bacterial lipids is well established[15,16] but, whereas in some species of bacteria certain of these constituents—namely the C_{15} and C_{17} iso and ante-iso acids[17–20]—represent major proportions of the total lipids, in ruminant body and milk fats these acids occur in trace amounts only. Akashi and Saito[17] postulated that branched-chain acids of animal fats[21,22] were derived from bacterial lipids in the gut of the host, and this hypothesis is supported by the investigation of Keeney *et al.*[13] on the rumen contents of a lactating cow, by the studies of Kuzdzal-Savoie[23] and by this report.

The chemical structure of phytanic acid, as distinct from that of the iso and ante-iso acids, suggests a close relationship with the isoprenoid alcohol phytol which constitutes a substantial proportion of the chlorophyll molecule of green plant life. An examination of the lipids extracted from the clover-grass hay fed to the experimental cow did not, however, reveal phytanic acid. It seems probable, therefore, that rumen bacteria convert phytol to phytanic acid within their own tissues and that the presence of this C_{20} tetramethyl fatty acid in ruminant body and milk fats is due to assimilation of the lipids of these micro-organisms[4].

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Effect of Boiling and Storing on Cycasin Content of *Cycas circinalis* L.

RECENTLY we described a quantitative chromatographic method[1], using the extraction procedure by Riggs and Korsch[2], for the specific estimation of cycasin in the kernel of *Cycas circinalis*. Matsumoto and Strong[3] have reported a chromotropic acid method which can be used for indirectly assessing cycasin from the quantity of formaldehyde liberated. Hydrolysis of cycasin by acid[4] or by its emulsin[5] is known to yield methanol[1], formaldehyde and nitrogen.

Our own method has been considerably simplified. First, the soaking and washing for 8 days through three changes of water (carried out initially to emulate the cooking procedure of the Guamanians)[6] has been dispensed with since this seems to remove the cycasin from the cycad nut[1,7]. Secondly, the Riggs and Korsch[2] procedure of extraction, which was followed initially and which involves boiling for 20 min, can also be dispensed with. The method now followed is reduced to: (1) cutting the cycad nuts into small slices; (2) drying the slices overnight in an oven at 40° C; (3) powdering of the dried slices; (4) extracting by stirring in 80 per cent ethanol for 15 min; (5) centrifuging at 3,000 r.p.m.; (6) directly spotting the clear supernate on Whatman No. 1 paper, using amounts equivalent to 25–50 mg of the original kernel; (7) unidirectional paper chromatography; (8) staining; and (9) colorimetric quantitation, as earlier. Recovery of known amounts of authentic cycasin is quite adequate with this method also.

Results obtained using this simplified procedure are given in Table 1, and compared with the cycasin content of the same specimen assessed by the chromotropic acid method. It is seen that on the whole the values obtained are comparable, especially in materials '*B*' and '*D*'. There appears to be a difference, however, in the values