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Characterisation of *N*-(2-hydroxyethyl)alanine as a component of a new phospholipid isolated from rumen protozoa

During a recent investigation of the ciliatine-containing phospholipids of rumen micro-organisms we obtained evidence of a new type of phospholipid which on hydrolysis gave an unknown ninhydrin-reacting neutral amino acid[1]. This latter substance has now been isolated and characterised as the *N*-(2-hydroxyethyl) derivative of alanine.

The preparation of the protozoa from rumen contents and the extraction of lipids were carried out as previously described[1]. The phospholipids were converted into their calcium salts by shaking their solution in chloroform–methanol (2:1, v/v) with 0.2 vol. of 0.1 M $CaCl_2$ and separating off the lower phase. This enabled a valuable preliminary purification of the new phospholipid to be made on a silicic acid column, since it ran differently from the sodium salt. The progress of the separation was monitored by paper ionophoresis of the deacylated phospholipid fragment[2]. It was eluted from the silicic acid column along with some phosphatidylinositol, phosphatidylethanolamine and pigmented material by chloroform–methanol (9:1, v/v). The eluted lipids were washed[3] with EDTA (sodium salt) solution (pH 7.5) to remove calcium and rechromatographed on silicic acid. On stepwise elution of the column with increasing concentrations of methanol in chloroform the sodium salt of the new phospholipid was eluted in chloroform-methanol (3:2, v/v). The new phospholipid gave a single spot when examined by thin-layer chromatography (silica gel H (Merck); chloroform–methanol–water–acetic acid (65:25:4:1, by vol.); chloroform–methanol–4.5 M ammonia solution (65:30:5.7, by vol.); detection by a phosphorus locating spray[4] followed by charring. Alkaline degradation gave a single deacylation product when examined by paper chromatography and ionophoresis[2].

The phospholipid was subjected to alkaline methanolysis (0.1 M NaOH in chloroform–methanol (1:1, v/v); 1 h; 20°), and the fatty acid methyl esters removed by distribution between chloroform and 50% aqueous alcohol. Alkali was removed from the aqueous alcohol layer by passage through Amberlite IRC 50 (H^+) and the eluate taken to dryness and the deacylated phospholipid fragment hydrolysed by heating in 1 M HCl at 100° for 2 h. The hydrolysate was reduced to dryness and the residue after dilution with water and adjustment to pH 2 with HCl, was passed through a column of Zeocarb 225 (H^+ form) to remove the nitrogen-containing component. The latter was then eluted from the column with 5 M NH_4OH. The eluate was taken to dryness and the product was crystallised from hot methanol. A sample of the new phospholipid containing 10 mg P yielded 7 mg of thin colourless needles of the nitrogen-containing component (I).

Examination of I by paper chromatography and ion-exchange chromatography indicated that it did not correspond to any of the ninhydrin-reacting substances which commonly occur in nature[5,6]. On a weight basis the ninhydrin colour on paper was weak compared with that given by the usual amino acids, and the acetaldehyde–nitroprusside test for secondary amines[7] gave a positive reaction. Strong acid hydrolysis (5 M HCl, 48 h, 110°) produced no further decomposition as judged by two-dimensional paper chromatography of the hydrolysate. The NMR spectra in ^2H_2O

suggested that the compound contained a CH_3CH-grouping separated from $-CH_2CH_2-$ by a hetero atom. Mass spectrograms of I untreated and after methylation and trifluoroacetylation were unhelpful. Eventually I was characterised as *N*-(2-hydroxyethyl)alanine by the following criteria. On analysis I gave C, 45.0; H, 8.4; N, 10.3; O, 36.3%; *N*-(2-hydroxyethyl)alanine ($C_5H_{11}O_3N$) requires C, 45.1; H, 8.3; N, 10.5; O 36.1%. Both I and authentic DL-*N*-(2-hydroxyethyl)alanine synthesized by the method of VIÈLES AND SÉGUIN[8] gave identical NMR spectra. They were inseparable on paper chromatography in eleven different solvent systems, and moved to identical positions (near to ethanolamine) on ion-exchange chromatography in an amino acid analyser.

As far as we are aware *N*-(2-hydroxyethyl)alanine has not previously been reported in nature. Linked to a phospholipid structure through a O–phosphate ester bond it would produce a hydrophilic head group which is acidic in nature. Since phosphatidylserine is absent from rumen protozoa[1] it is possible that the new phospholipid substitutes for this in the membranes of these micro-organisms.

Biochemistry Department P. KEMP
A.R.C. Institute of Animal Physiology, R. M. C. DAWSON
Babraham, Cambridge (Great Britain)

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Oxidation of *n*-hexadecane by mouse liver microsomal fraction

MAHDI AND CHANNON[1] and CHANNON AND DEVINE[2] were the first to suggest that mammals can catabolize *n*-hexadecane (see also ref. 3). Recently, MCCARTHY[4] demonstrated that [1-^{14}C]hexadecane and [1-^{14}C]octadecane were converted directly to the corresponding fatty acids *in vivo* in goat, rat and chicken, and also in liver homogenates from goat and rat. However, little information is available concerning the nature of the oxidation of long-chain aliphatic hydrocarbons at a subcellular level except for a preliminary report of MITCHELL AND HÜBSCHER[5]. During the course of our investigation on microsomal ω-oxidation of fatty acids, we have also found that the microsomal fraction of mouse liver contains an enzyme system hydroxylating long-chain aliphatic hydrocarbons requiring NADPH and oxygen[6].

Male ddO mice 6 weeks of age were used in this study. The mice were killed