221° (partially melted at 178° and resolidified, which showed only one yellowish green spot with $SbCl_3$ on paper chromatography. It showed identical behavior as deacetylmetaplexigenin from *Metaplexis japonica* and a mixed melting point showed no depression. The IR spectra also confirmed the identity of both sample. The acetate, m.p. 262~263°, prepared according to the usual procedure was also shown to be identical with a sample of acetylmetaplexigenin by mixed melting point and IR spectra.

Cryst. A_4 (=Isodeacylcynanchogenin)——The mother liquors from the hydrolysate of the cynanchogenin fraction were submitted to partition chromatography over Celite (100 g.) containing H_2O (100 ml.). The results are shown in Table II.

Table II. Partition Chromatography of A_4 Fraction

Fr. No.	Solvent	Note	Weight (mg.)	Spot on paperchromatography
1~10	Be	oil	25.2	—
11~23	Bu-Be (5:95)	〃	15.0	—
24~34	〃 (3:97)	cryst.	64.3	A_4
35~40	〃 (3:97)	〃	58.0	〃
41~45	〃 (3:97)	oil	10.0	A_4, DC
46~60	〃 (1:6)	cryst.	190.0	DC
61~62	〃 (1:4)		trace	

1 fraction 20 ml.

Fraction 24~40 were collected and recrystallized from acetone to give needles, m.p. 248~249.5° which was pure isodeacylcynanchogenin. It is noteworthy that deacylcynanchogenin gave isodeacylcynanchogenin (A_4) on paper chromatography after alkaline treatment. *Anal.* Calcd. for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85. Found: C, 68.64; H, 8.74.

The authors are indebted to Mrs. T. Toma of this Faculty for micro analysis.

Summary

The alkaline hydrolysate of the crude aglycones of *Cynanchum caudatum* Maxim. showed the existence of two new aglycones, besides deacylcynanchogenin and sarcostin, by paper chromatography. The one (A_3) was shown to be deacetylmetaplexigenin, and the other (A_4) as isodeacylcynanchogenin. A_4 appears to be an isomer of deacylcynanchogenin formed under the conditions of alkaline hydrolysis.

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Makoto Hayashi, Yoshinori Nakajima, Keizo Inoue, and Komei Miyaki :
Determination of Threonine in Lipid of Animal Brain.

(*Institute of Food Microbiology, Chiba University*[*1])

It has bees reported by Rodes,[1] Igarashi[2] and Wallach,[3] *et al.* that threonine is contained in lipid, especially in phospholipid fraction. However, quantitative studies of this subject have not been published. Therefore, the authors analysed quantitatively threonine in lipid of animal brain, establishing the chemical method for the estimation of small amount of threonine.

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1) D.N. Rodes, C.H. Lea : Biochem. J., **65**, 526 (1957).
2) H. Igarashi, K. Zama, M. Katada : Nature, **181**, 1282 (1958).
3) D.F.H. Wallach, J. Sodergerg, C. Bricker : Cancer Research, **20**, 397 (1960).

Experimental

Extraction and Partial Purification of Lipid——3 to 5 g. of brain was removed from mouse, rat, guinea pig and rabbit as soon as they were killed. This was extracted with ten times quantity of $CHCl_3$-MeOH (2:1) by homogenation and then filtrated. The residue was extracted in the similar manner repeatedly three times. All of extracted filtrates were combined and dried under reduced pressure in N_2 atmosphere. In order to remove contaminated amino acids and peptides, the dried material was dissolved into 10 ml. of $CHCl_3$. Soluble part was passed through cellulose column (where ca. 8 g. cellulose powder was used to 100 μmoles of lipid-P). Cellulose column was further washed with 50 ml. of $CHCl_3$, which is added to the above filtrate. This filtrate was dried under reduced pressure in N_2 atmosphere. In this case, it was confirmed by paper chromatography that amino acids and peptides were removed. A part of solution was used for the analysis of lipid-P and other most part was hydrolysed.

Hydrolysis of Lipid——The definite quantity (ca. 200 μmoles of total lipid-P) of $CHCl_3$ solution of lipid, which was purified partially, was placed into a tube of pressure resisting glass. By distilling off $CHCl_3$ gently in a water bath, lipid was precipitated at the bottom wall of the pressure resisting tube in the state of thin film. Then, 10 to 20 ml. of 6*N* HCl was added gently into the tube and the content was hydrolysed at 110° to 120° for 4 hr. (more than 95% is hydrolysed under this condition). From hydrolysed solution, unsaponified lipid and fatty acid were extracted three times with 5 ml. of Et_2O. All of Et_2O layers were washed once with 5 ml. of H_2O and washing solution was combined with the hydrolysed solution. This solution was dried under reduced pressure. In order to remove excess HCl, the dried material was kept in a desiccator under reduced pressure containing KOH for 1 night. This was dissolved into 5 ml. of distilled H_2O and used as the sample for analysis.

Fractionation of Serine, Threonine, Ethanolamine and Choline——According to the method of Hayashi, *et al.*,[4] fractions of serine, threonine, ethanolamine and choline were obtained by Dowex 50-X2 ion exchanger column chromatography. Chromatographic patterns of serine, threonine, ethanolamine and choline are shown in Fig. 1.

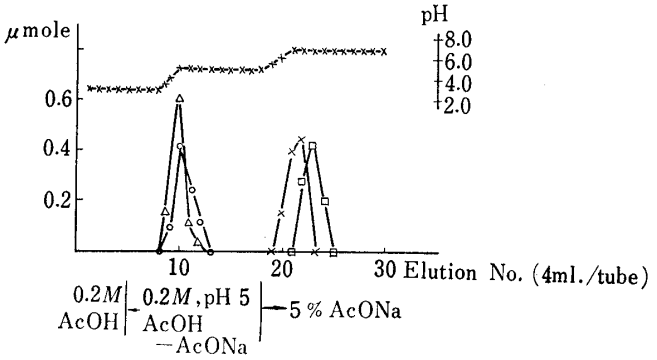

Fig. 1. Chromatographic Pattern of Serine, Threonine, Ethanolamine and Choline

Amino acids and amines were used 1.0 μmole respectively.

o—o serine
△—△ threonine
×—× ethanolamine
□—□ choline

Method of Quantitative Analysis——Lipid-P was estimated by the method of Allen.[5] Serine and ethanolamine were analysed by the method of Hayashi, *et al.*[4] Choline was analysed by the method of Hayashi, *et al.*[6]

Since threonine appeared in the same fraction of serine as shown in Fig. 1, acetaldehyde and formaldehyde were produced by periodate oxidation. The reaction of chromotropic acid to formaldehyde is specific but 4-hydroxybiphenyl used for the estimation of acetaldehyde is a common coloring reagent for aldehydes. Therefore, quantitative analysis of acetaldehyde under the coexistence of formaldehyde was studied. The following method was established from the facts that the diffusion rate of acetaldehyde from aqueous solution is higher than that of formaldehyde and that the latter can be trapped easier than the former by the addition of glycine. That is, just before the measurement, 1 ml. of 3% $NaHSO_3$-H_2O solution, pH of which was adjusted to 7.5 by NaOH, was introduced into the center well of Conway micro-diffusion unit No. 1, and 1 ml. of sample, 0.5 ml. of 0.5*M* Na_2HPO_4, 0.5 ml. of *M* glycine and 0.5 ml. of 0.05*M* HIO_4 were introduced into the outer well. The lid was put on and kept at room temperature for 2 hr. Then 0.5 ml. of sample was taken from the center well. To this aliquot, 0.5 ml. of H_2O, one drop of 4% $CuSO_4$ and 6 ml. of conc. H_2SO_4 were added. After well stirring and

4) M. Hayashi, T. Unemoto, K. Miyaki: This Bulletin, **8**, 904 (1960).
5) R. T. Allen: Biochem. J., **34**, 858 (1940).
6) M. Hayashi, T. Unemoto, K. Miyaki: This Bulletin, **10**, 533 (1962).

cooling in an ice bath, 0.2 ml. of 1.5% 4-hydroxybiphenyl (in 2 to 4% NaOH) was added to the mixture. This was reacted at 30 to 37° for 30 min. and then heated at 100° for 90 sec. to dissolve excess 4-hydroxybiphenyl. After cooling, this was used for quantitative colorimetry at 580 mμ. Under this condition, the coexistence of ca. fifty times of serine to minimum quantity (0.06 μmole per tube) of threonine for quantitative analysis has no effect.

Result

Obtained results were shown in Table I. From this table, it is obvious that threonine exists in brain lipid of mouse and rat in the same quantity but it is not detected in brain lipid of guinea pig and rabbit. Although a question "In what type threonine exists as lipid ?" is not clear, this seemed to be interesting from the viewpoint of comparative biochemistry.

Table I. Contents of Threonine, Serine, Ethanolamine and Choline in Brain Lipid of Mouse, Rat, Guinea-pig and Rabbit

	Threonine	Serine	Ethanolamine	Choline
	(μ moles per 10 μ moles of lipid-)			
Mouse	0.040±0.002	1.0±0.09	3.4±0.40	4.3±0.29
Rat	0.041±0.003	1.1±0.12	3.1±0.28	4.1±0.32
Guinea-pig	none	1.3±0.09	3.1±0.30	4.1±0.27
Rabbit	〃	1.5±0.013	3.3±0.32	4.0±0.30

Summary

Quantitative analysis of threonine in brain lipid of various animals was performed and it was found that brain lipid of mouse and rat containes 0.04 μmole of threonine per 10 μmoles of lipid-P, while that of guinea-pig and rabbit does not contain threonine at all.

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Yuriko Kato : Formation of a Micelle-like Structure in Aqueous Solution of 1,4-Hexanediol.

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In the previous paper[1] it was reported that physical constants were measured of aqueous solution of fourteen kinds of glycols of different number of carbon atoms, from propanediol to dodecanediol. The following results were obtained.

These experimental results show that glycols with hydroxyls on either terminal ends or in 1, 2-positions were considered to form a micelle-like structure. Glycols with one hydroxyl at a terminal end and the other in a median position also forms a micelle-like structure but those having both hydroxyls in median positions and none at the terminal position do not form such a structure.

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1) Y. Kato : This Bulletin, **10**, 771 (1962).