(−)-maalian-5-ol **(I)** gave opposite sign compared with the values ($[\alpha]_D$ −138° and $[\alpha]_D$ +149°) of the known compounds[4].

Accordingly, it was certain that the alcohol consists of the structure and absolute configuration, excluding the tertiary hydroxyl group on C-5, as shown in the stereostructure **I**. Next, the configuration of the hydroxyl group was elucidated by examination of the solvent effect on ^{1}H-NMR spectra to be trans to both the methyls on C-4 and C-10: when the ^{1}H-NMR spectra of the alcohol **(I)** were measured in $CDCl_3$ and C_5D_5N solutions, the C-4 secondary methyl (Δ 0.04) and the C-10 tertiary methyl (Δ 0.02) exhibited no solvent shift due to vicinal deshielding of the C-5 hydroxyl group[5].

On the basis of the above chemical and spectroscopic evidence, the structure and absolute configuration of the sesquiterpene alcohol, (−)-maalian-5-ol, was determined to be shown by the stereostructure **I**. This may be biosynthesized from trans-farnesyl pyrophosphate via (−)-bicyclogermacrene which has been isolated as a common component in the liverworts[6]. We were interested in such occurrence of the enantiomeric sesquiterpenoids in the liverworts with respect to a chemotaxonomy of the plants and a biogenesis of the compounds.

1 The authors express their gratitude to Dr H. Inoue, National Science Museum, for identification of the liverwort, and Prof. T. Tokoroyama, Osaka City University, for sending of the data of (−)-β-maaliene and (+)-selina-5,11-diene. Thanks are also due to the Ministry of Education for financial support of this work.

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6 At our laboratory (−)-bicyclogermacrene were isolated from the following liverworts, *Porella densifolia, Plagiochila semidecurrens, Lepidozia vitrea, Riccardia jackii*, etc. (unpublished data).

Biochemical compartmentation of fish tissues. II. Nonspecific phosphomonoesterases in brain

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Summary. The specific activities of acid and alkaline phosphatases in different regions of the brain of 9 nutritionally important fishes were worked out. The region consisting of pituitary, hypothalamus and thalamus showed the highest acid and alkaline phosphatase activities. Least activities of both the enzymes were found in the cerebellum and medulla oblongata. The piscivorous fishes contain the highest acid and alkaline phosphatase activity followed by cat fishes and major carps. The distributional pattern of these 2 enzymes in 4 regions of the brain of 9 fishes is the same.

Recently, studies in the biochemical aspect of animal tissues has been encouraged for a better insight into biochemical organisation[2-4]. Much attention has been paid in the past to the mammalian brain with regard to key enzymes like glutamate de-carboxylase, choline acetyl, transferase, glucose-6-phosphatase, fructose 1, 6-diphosphatase, important neurotransmitters like catecholamines, energetic substances like adenine-nucleotides, glycogen, glucose, lactate and pyruvate[5-10]. However, the compartmentation of the brain in fishes has to be investigated. It is known that various parts of the brain differ in their biochemical make-up following distinct phylogenetic ages.

Although acid and alkaline phosphatases are widely distributed in animal tissues, they are mainly concerned with the reabsorbtion of glucose molecules in renal tissue, to conduct an active glycolytic mechanism in liver, dephosphorylation of glucoesters in sex organs, active involvement in protein synthesis, association with the secretory activity and absorption of nutrients in digestive system, phosphate cycles and growth[11-13]. However, the functional significance of these phosphatases in various regions of the brain is still to be completely known. If we want to understand fully the complex functions of the brain as a central, controlling, regulating and co-ordinating organ for sensory and memory functions, it is essential to know the levels of the nonspecific phosphomonoesterases in different regions of brain, because of the close relation between brain metabolism and neuronal activity[14].

The functional significance of alkaline phosphatase (orthophosphoric monoester phosphohydrolase EC 3.1.3.1) in brain is supposedly involved in various secretory and transport processes and in blood-brain barrier mechanisms[14]. The acid phosphatase (orthophosphoric monoester phosphohydrolase EC 3.1.3.2) is implied again in the secretary processes and in the intracellular transport mechanisms. Such non-specific phosphomonoesterases are also involved in transphosphorylation[3]. The main aim of the present investigation is to study the differential distribution and functional role of these phosphatases in 4 regions of the brain i.e. cerebrum, cerebellum, the region consisting of pituitary, hypothalamus and thalamus and medulla oblongata of 9 nutritionally and economically important fishes i.e. *Labeo rohita* (Ham), *Cirrihina mrigala* (Ham), *Catla catla* (Ham) (herbivorous and soft fishes), *Channa punctatus* (Bloch), *Channa marulius* (Ham), *Channa striatus* (Bloch) (hard and piscivorous) and *Clarias batrachus* (Linn), *Heteropneustes follilis* Bloch and *Mystus seenghala* (sykes) (hard and carnivorous). This is a first

attempt to investigate fish from a tropical environment on a comparative basis.

Materials and methods. Healthy *L. rohita, C. mrigala, C. catla, C. punctatus, C. marulius, C. striatus, C. batrachus, H. fossilis* and *M. seenghala* of a particular size range (18-20 cm, 10-month-old) were obtained locally from some selected ponds to avoid the ecological variations, and acclimatized to the laboratory immediately. The major carps *(L. rohita, C. mrigala* and *C. catla)* were fed with algae, decayed higher plants, vegetable debris, cat fishes *(C. batrachus, H. fossilis* and *M. seenghala)* with worms, insects, decayed organic matter and murrels *(O. punctatus, O. streatus* and *O. marulius)* and with small and medium-sized fishes (*Trichogaster, Barbus* and *Chela* sps.). Water was brought from the selected ponds (from where the fishes were obtained) to provide a natural environment to the fishes in terms of physico-chemical parameters in the laboratory aquaria, and kept for a period of 2 days[13]. The size of the aquaria is $5 \times 1.5 \times 1.5$ m. The water in the aquaria was changed every 10 h. Fishes were killed by decapitation and the brain was carefully removed as quickly as possible, and then separated into cerebrum, cerebellum, the region consisting of pituitary, hypothalamus and thalamus and medulla oblongata by the method described earlier[8]. The preparation of tissue samples and enzymes estimations were the same as described elsewhere[16]. The experiment was repeated 6 times, student's t-test (paired) was performed to know the level of significance.

Results and discussion. It is evident from table 1 that marked differences exist in the acid and alkaline phosphatase activities among the 4 regions of the brain of these 9 nutritionally important fishes. The highest acid and alkaline phosphatase activity was found in the region consisting of pituitary, hypothalamus and thalamus followed by cerebrum. The lowest values were recorded in medulla oblongata and cerebellum. Among these 3 groups of fishes, the piscivorous and snake-headed fishes (table 2) contain the highest acid and alkaline phosphatase activity *(C. punctatus, C. striatus, C. marulius)* followed by cat fishes which are carnivorous (table 3) (*C. batrachus, H. fossilis* and *M. seenghala)* and major carps which are herbivorous *(L. rohita, C. mrigala* and *C. catla)* (table 1). The presence of these 2 enzymes indicate their metabolic involvement in different compartments of the brain. If the biochemical picture of the different regions of the brain are available, it may help to find some mechanisms to cure acute and chronic diseases of the brain.

Table 1. Differential distribution of acid and alkaline phosphatase in different regions of the brain - majors carps

Name of the fish	Regions of the brain	Enzyme activity (μg of pi/mg protein at 37 °C) Acid phosphatase	Alkaline phosphatase
L. rohita	Cerebrum	$0.055^{d} \pm 0.004$	$0.091^{d} \pm 0.007$
	Cerebellum	0.034 ± 0.005	$0.051^{d} \pm 0.009$
	Pituitary, thalamus and hypothalamus	0.108 ± 0.008	0.173 ± 0.012
	Medulla oblongata	$0.043^{b} \pm 0.006$	0.065 ± 0.009
	Reference value*	0.075 ± 0.009	0.110 ± 0.006
C. mrigala	Cerebrum	$0.042^{d,b} \pm 0.002$	$0.074^{b,d} \pm 0.007$
	Cerebellum	$0.025^{d} \pm 0.005$	$0.045^{d} \pm 0.010$
	Pituitary, thalamus and hypothalamus	0.096 ± 0.006	0.138 ± 0.014
	Medulla oblongata	0.031 ± 0.008	0.058 ± 0.005
	Reference value*	0.052 ± 0.004	0.089 ± 0.005
C. catla	Cerebrum	$0.032^{d} \pm 0.008$	$0.062^{d} \pm 0.008$
	Cerebellum	$0.020^{d} \pm 0.005$	$0.034^{d} \pm 0.004$
	Pituitary, thalamus and hypothalamus	0.066 ± 0.008	0.113 ± 0.006
	Medulla oblongata	0.022 ± 0.006	0.037 ± 0.003
	Reference value*	0.039 ± 0.004	0.066 ± 0.007

Values are mean ± SEM of 6 replicates. Student's t-test was performed between specific activities of acid and alkaline phosphatases in different regions of the brain. Superscripts a-d indicate that $p > 0.05$. * Whole brain homogenate values.

Table 2. Differential distribution of acid and alkaline phosphatase in different regions of the brain - snake headed fishes

Name of the fish	Regions of the brain	Enzyme activity (μg of pi/mg protein at 37 °C) Acid phosphatase	Alkaline phosphatase
C. punctatus	Cerebrum	0.156 ± 0.007	0.323 ± 0.024
	Cerebellum	0.117 ± 0.008	0.196 ± 0.014
	Pituitary, thalamus and hypothalamus	0.284 ± 0.017	0.527 ± 0.029
	Medulla oblongata	0.183 ± 0.004	$0.269^{a} \pm 0.010$
	Reference value*	0.220 ± 0.010	0.364 ± 0.020
C. striatus	Cerebrum	$0.121^{b} \pm 0.017$	0.261 ± 0.014
	Cerebellum	0.099 ± 0.008	0.151 ± 0.015
	Pituitary, thalamus and hypothalamus	$0.216^{a} \pm 0.013$	0.440 ± 0.031
	Medulla oblongata	$0.150^{a} \pm 0.012$	$0.201^{a} \pm 0.009$
	Reference value*	0.164 ± 0.009	0.291 ± 0.015
C. marulius	Cerebrum	0.104 ± 0.008	$0.211^{d} \pm 0.014$
	Cerebellum	$0.075^{a} \pm 0.012$	0.135 ± 0.007
	Pituitary, hypothalamus and thalamus	0.184 ± 0.011	0.343 ± 0.013
	Medulla oblongata	$0.118^{a} \pm 0.007$	0.184 ± 0.011
	Reference value*	0.132 ± 0.005	0.236 ± 0.011

Values are mean ± SEM of 6 replicates. Student's t-test was performed between specific activities of acid and alkaline phosphatases in different regions of the brain. Superscripts a-d indicate that $p > 0.05$. * Whole brain homogenate values.

Table 3. Differential distribution of acid and alkaline phosphatase in different regions of the brain - cat fishes

Name of the fish	Regions of the brain	Enzyme activity (μg of pi/mg protein at 37 °C) Acid phosphatase	Alkaline phosphatase
H. fossilis	Cerebrum	0.120 ± 0.005	0.185 ± 0.007
	Cerebellum	0.081 ± 0.009	0.116 ± 0.008
	Pituitary, thalamus and hypothalamus	0.173 ± 0.005	0.287 ± 0.010
	Medulla oblongata	0.109^{a-b} ± 0.006	0.137^{b} ± 0.007
	Reference value*	0.146 ± 0.006	0.206 ± 0.012
C. batrachus	Cerebrum	0.097 ± 0.004	0.149 ± 0.008
	Cerebellum	0.070 ± 0.007	0.097 ± 0.006
	Pituitary, thalamus and hypothalamus	0.145 ± 0.013	0.248 ± 0.015
	Medulla oblongata	0.081^{a-b} ± 0.017	0.124^{a-b} ± 0.011
	Reference value*	0.116 ± 0.005	0.172 ± 0.009
M. seenghala	Cerebrum	0.081 ± 0.008	0.127 ± 0.011
	Cerebellum	0.060^{a} ± 0.007	0.081 ± 0.009
	Pituitary, thalamus and hypothalamus	0.133 ± 0.014	0.209 ± 0.010
	Medulla oblongata	0.066^{a-b} ± 0.012	0.104^{a-b} ± 0.008
	Reference value*	0.095 ± 0.008	0.140 ± 0.010

Values are mean ± SEM of 6 replicates. Student's t-test was performed between specific activities of acid and alkaline phosphatases in different regions of the brain. Superscripts a-b indicate that $p > 0.05$. * Whole brain homogenate values.

The region consisting of pituitary, hypothalamus and thalamus not only assumes a greater significance as a coordinating centre and a source of different hormonal secretions like the ones involved in reproduction, but also indicates their participation in various secretory, transport, and blood-brain barrier mechanisms due to their highest acid and alkaline phosphatase activities. The cerebrum, which is considered to be the organ of sensation, has the 2nd highest acid and alkaline phosphatase levels. Although, cerebellum and medulla oblongata which are intensively involved in neuronal activity and glycolysis respectively, showed lowest levels of acid and alkaline phosphatases. Regions of the brain belonging to lesser hierarchy displayed lesser phosphomonoesterase activities.

The reasons for the differential distribution of acid and alkaline phosphatases are not clearly known at present. It may be explained on the basis of classification. The major carps which are primitive (table 1), occupy first place in the order cypriniformes (group cyprini) then cat fishes (group siluri table 3) which come under the same order. The snake-head fishes, which are advanced, come in another order (channiformes, table 1) which is far away from cypriniformes in classification. The author observed a direct relationship between the course of evolution and the rise in the levels of phosphatases. The phylogenetic older fishes (major carps, table 1) contain lower levels of phosphatases than the phylogenetic younger animals (cat fishes and snake-headed fishes, tables 3 and 4). Even the phylogenetically older parts of the brain (medulla oblongata) contain lower levels of phosphatases than the younger parts (pituitary, hypothalamus and thalamus) of the brain. This may be due to intensive glycolytic activity in phylogenetically older parts of the brain (medulla oblongata)[4] and it may be an important biochemical adaptation among different regions of the brain as one part of the brain may be intensively involved only in one type of metabolism rather than all types of metabolism. Secondly, it may be related with the diet. The highest acid and alkaline phosphatase activity in snake-headed fishes (table 2) may be related with the piscivorous feeding habit, and the lowest in cat fish (table 3) and major carps (table 1) may be related with carnivorous and herbivorous diets respectively. These 2 interpretations may be the possible reasons for the differential distribution of phosphatases in 4 regions of the brain of 3 groups of fishes. From the above results and discussion, it is suggested that phylogenetic age has an influence on fish in general and the organ in particular. This is true with acid and alkaline phosphatase in different regions of the brain of the 9 fishes tested. The content of energy reserve (glycogen) and its metabolic products (lactate and pyruvate), gluconeogenic enzymes and adenine nucleotides (ATP, ADP and AMP) were biochemically compartmentalised in tissues of primitive fishes (major carps) and they are higher than that of the advanced fishes (cat fishes and murrels)[17]. This also supports again the interpretation that phylogeny has an influence on fish in general, and the organ in particular in relation to biochemical compartmentation of the tissues. The possibility of their varied associations in the phosphate cycles and nerve action have already been suggested[11].

1 The author is grateful to Prof. J.S. Rajput for encouragement and to Dr C.K. Ashok Kumar for critically going through the manuscript.
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