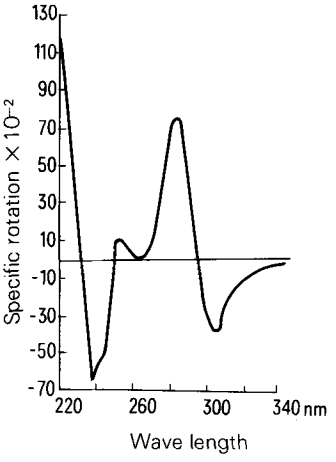

Optical rotatory dispersion curve (in ethanol) of attenuol (**I**).

From the generalized observations of Swan and Klyne[6] on the stereochemistry of 1-aryl tetralins, since attenuol shows the molecular amplitude ($a \times 10^{-2}$) of $-347°$, the substituents of ring B should be 1S, 2S and 3R. Thus the new lignan should have the stereostructure (**III**) in which H-2 and H-3 are trans to each other. All the substituents in ring B are pseudo-equatorial.

In its mass spectrum attenuol (**I**) showed the molecular ion peak at m/e 296 (58%) and a prominent fragment arizing from reverse Diels-Alder reaction of ring B[7] giving the ion (a) followed by cycloaddition to the ion (b). Other fragments which could be attributed to the ions (c) and (d) are also observed.

Closely related lignans of this type have been isolated from plants belonging to the Myristicaceae family, e.g. otobain and otobaphenol from *Myristica otoba*[8]. Biogenetically it is well known that such lignans are formed by the dimerization of C_6-C_3 precursors (generally cinnamyl alcohols).

It is of interest to note that attenuol is the only example of a 1-aryl lignan in which the ring C contains only 1 oxygen substituent.

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A new alarm pheromone (2-decen-1-yl-acetate) isolated from the stings of *Apis dorsata* and *Apis florea* (Hymenoptera: Apidae)

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Summary. From sting extracts of *Apis dorsata* and *A. florea*, a substance was isolated which is active in alarm behaviour of both of these species but not of *A. mellifera* and *A. cerana*. The active substance was identified as 2-decen-1-yl-acetate.

Isopentyl acetate has been isolated from the sting apparatus of honey bee workers in 1962[2]. This pheromone evokes a typical alarm behaviour from bees guarding the entrance of their hive. Morse et al. found that also the stings of *A. dorsata*, *A. cerana* and *A. florea* contain isopentyl acetate[3]. However, comparative tests showed that a freshly prepared sting apparatus of *A. florea* is longer effective than one of *A. mellifera* and *A. cerana* although the latters have more isopentyl acetate in their stings. A fraction - containing no isopentyl acetate - isolated from the sting extracts of *A. dorsata* and *A. florea* was active in the bioassays of both of these species but not of *A. mellifera* and *A. cerana*[4]. In this communication the analysis of the active compound is reported.

Material and methods. *A. mellifera* was of the carnica-race, *A. dorsata*, *A. cerana* and *A. florea* originated from Sri Lanka (Anuradhapura and Kandy). These species were kept in tropical flight-rooms; thus we always could work with freshly prepared material. For preparation the bees were caused to sting into a soft piece of leather, stings were plucked with forceps and dropped into a centrifuge tube immersed in dry ice. To the stings (100 per tube and species) 1 ml of n-pentane was added, tube removed from dry ice, the contents macerated and centrifuged briefly.

The products were analyzed by GC-MS; gas chromatograph Perkin-Elmer F20 equipped with a 3.5 m glass column, 0.1% Carbowax 20 M on glass beads (60-80 mesh), column temperature programmed from 70 °C to 200 °C (20 °C/min). The gas chromatograph was coupled to a Varian-Mat CH4-B mass spectrometer which was connected to a Varian-Mat SS100 MS data system.

Results and discussion. The sting extracts of *A. dorsata* and *A. florea* mainly contain the esters of acetic acid with higher alcohols. In the mass spectra of acetates, the base peak is due to the acetylium ion (m/e 43). The ion at m/e 61 results from a double hydrogen transfer and corresponds to the protonated acetic acid[5]. The mass spectra of acetates with higher alcohols exhibit no molecular ion peak under electron impact conditions. Mass highest peaks are recorded for the ions $M-60^+$ and $M-42^+$ e.g. elimination of acetic acid and ketene from the molecular ion.

In the gas chromatograms of the sting extracts of *A. dorsata* and *A. florea*, 2 peaks are registered with higher intensities, besides the peak for isopentyl acetate. The compound with longer retention time could be identified as 2-decen-1-yl-acetate[6]. The retention time and the mass spectrum was identical with that of a synthetic product (m/e 156 (M-42), 5%; m/e 138 (M-60), 9%; m/e 43, 100%). The synthetic sample was prepared by a Reformatzky reaction with ethyl-α-bromoacetate and caprylaldehyde[7], dehydration with

H_3PO_4, reduction of the ester function with $LiAlH_4$ and acetylation with acetic anhydride in pyridine. The product was purified by preparative column chromatography. Synthetic 2-decen-1-yl-acetate was active in the bioassays of *A. dorsata* and *A. florea* but not of *A. mellifera* and *A. cerana*.

The 2nd peak in the gas chromatogram of an unknown substance could be identified as 1-octyl acetate. 1-Octyl acetate is not at all active in all species of genus *Apis*, independent of its concentration or mixed with other substances.

Colony defence of *A. mellifera* and *A. cerana* differs from that of *A. dorsata* and *A. florea* which are free nesting forms. The latter species are able to mark intruders by their stings so that these are pursued over long distances and recognized even after 1 h[4]. Responsible for this behaviour is 2-decen-1-yl-acetate; with its low vapor pressure, it serves as an enemy-marking substance which is effective for much longer than isopentyl acetate as a highly volatile compound can be. By this additional alarm pheromone and by their better developed lancet barbs[8], *A. dorsata* and *A. florea* seem to be well adapted for the defence of their exposed colonies.

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Distribution of monoamine oxidase in hippocampal region of the rat

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Summary. The distribution and development of type A and type B monoamine oxidase (MAO) activities in the hippocampal region of the rat was investigated with biochemical microdetermination. Type A MAO is absolutely dominant and unevenly distributed in the hippocampus. The development of type A MAO in the hippocampus seems to be delayed and reachs adult levels by the 30th day after birth.

The regional distribution of monoamine oxidase (MAO) [EC 1.4.3.4.] activity has been measured in the rat hypothalamus and limbic system[1-3]. Consequently, the difference of distribution and development between type A MAO and type B MAO was noted in hypothalamic nuclei of the rat[4]. In regard to the histochemistry of MAO in the hippocampal region, detailed description was made for the guinea-pig[5-7] and the rat[8]. According to these studies, some differences of the staining pattern of MAO in the hippocampal region were detected between the species. However, regional distribution of MAO activity in hippocampal structure has not been studied biochemically, except for brief comments by Uchimura and others[9]. In the present paper, the distribution and the development of type A and type B MAO activity in the hippocampal region of the rat was investigated with biochemical microdetermination.

Materials and methods. Male Wistar rats of 15 and 30 days of age were used, as well as adult animals of 4 months of age. All animals were killed by decapitation at 09.00 h and the brains were immediately taken out and placed on ice. The blocks including the hippocampus were isolated and frozen quickly in liquid nitrogen. Frontal serial sections of 60 µm thickness were made in a cryostat at −15 °C. The sections were freeze-dried over 12 h at −30 °C and 5×10^{-4} mmHg and stored in evacuated tubes at −20 °C until use. 8 locations in the regio inferior of the hippocampus (figure) were dissected out of the freeze-dried sections with a microknife and a thin steel needle under a stereomicroscope. After being weighed with an electron microbalance (Type 4215, Sartorius Co.), each sample was placed into a pointed microtube. The sensitivity of this balance is 0.1 µg. The weight of each sample was 1.0–2.5 µg.

MAO was assayed by a previously described modification[1] of the method of McCaman et al.[10]. 2 µl of 0.1 M phosphate buffer, pH 7.2, containing BSA and Triton X-100 in each final concentration 0.05% were added to the weighed sample in a microtube. After a 20 min preincubation at 0 °C, 2 µl of ice-cold substrate buffer solution were added. Dopamine (DA) and 5-hydroxytryptamine (5-HT) were used as substrate for type A and B and type A MAO, respectively (final concentration of the substrate: 1.0 mM [2−^{14}C] dopamineethylamine, 60 mCi/mM, 1.1 mM [2-^{14}C] 5-hydroxytryptamine binoxalate, 45 mCi/mM, New England Nuclear Co.). After an incubation at 38 °C for 45 min, the reaction products were extracted in ethyl acetate and the radioactivity was measured by a liquid scintillation spectrometer as described elsewhere[1]. When the assay of type B MAO, 1 µl of 0.1 M phosphate buffer, pH 7.2, and 1 µl of 10^{-5}M clorgyline solution were added in a microtube, and after a preincubation at 0 °C for 20 min, 2 µl of the ice-cold DA-buffer solution (the same solution that was used for the assay of type A and B MAO) was added. In our preliminary experiment under this condition, the inhibition of DA and 5-HT deamination was found to be approximately 40% and 96%, respectively[4]. The inhibition of DA deamination with clorgyline showed plateau at the concentration of clorgyline between 10^{-6}M and 5×10^{-5}M. Accordingly MAO activity for DA in this condition was almost considered to mean type B MAO activity. Moreover, it was ascertained in this experiment that the enzyme reaction towards DA and 5-HT as substrate proceeded linearly with or without the inhibitor with the use of the freeze-dried rat cerebral cortex as enzyme sample between 0.5 µg and 8 µg in weight.

Results and discussion. The results are summarized in the table. As compared with the results on the hypothalamus[4], the hippocampus exhibited slightly higher MAO activity than the hypothalamus when DA was used as substrate. On the other hand, the hippocampus exhibited much lower activity of MAO towards 5-HT than the hypothalamus. According to MAO measurement with 5-HT, the hippocampus showed activity as low as the cerebral cortex[3]. In