trifugation at 13,000 *g* for 20 min. Protein in the supernatant fraction of the liver and serum was determined colorimetrically by the method of LOWRY et al.[10] with crystalline bovine serum albumin as a standard.

Results and discussion. As shown in Figure 1, the protein concentration of the serum of rats with chronic hepatic damage was lower than that of healthy rats, and no significant difference of the protein concentration in the liver was seen between healthy and hepatic damaged rats. By the administration of methylcobalamin for 7 days, a slight elevation, but not statistically significant, was observed in the protein concentrations of the liver and serum both of the healthy and chronic hepatic damaged rats, as shown in Figure 1. In healthy rats with partial hepatectomy, methylcobalamin administration showed no influence on the content of protein in the liver and serum as given in Figure 2. On the contrary, as shown in Figure 3, after the hepatectomy of rats with chronic hepatic damage, the protein concentrations in the liver and serum of the group administered methylcobalamin were significantly higher than those of the control group on the 7th postoperative day. The administration of cobamide (5,6-dimethyl-benzimidazolyl-5′-deoxyadenosyl cobamide) showed, in this condition, no effect such as seen by the methylcobalamin administration. This effect of methylcobalamin apparently seems to enhance protein synthesis in the process of regeneration of the liver with chronic hepatic damage, and it is assumed from these results that methylcobalamin accelerates the liver regeneration.

Zusammenfassung. Die Zunahme der Leber- und Serumeiweissmenge bei chronischer Leberstörung von Ratten mit partiel extirpierter Leber wird nach Applikation von Methylcobalamin gefördert. Eine Methylcobalamin induzierte Förderung der Eiweißsynthese in der Leber wird angenommen.

T. KOIZUMI, T. SUEMATSU, K. HIRAMATSU and H. ABE

Department of Medicine, Osaka University Medical School, Osaka 553 (Japan), 28 July 1969.

[10] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).

Strychnine Antagonistic Potency of Pyrone Compounds of the Kavaroot (*Piper methysticum* Forst.)

Up to now several aromatically substituted δ-lactones (formulas see [1]) have been isolated from the root-stock of the kavaplant (*Piper methysticum* Forst.), indigenous to many islands of the South Pacific. These compounds, the so-called kavapyrones, mainly act in pharmacological experiments as central depressant drugs. In detail we have found: depression of spontaneous motor activity, potentiation of anaesthesia[2], anticonvulsive effects against electroshock and pentylenetetrazol induced seizures[3], and, in particular, a centrally caused muscle relaxation as well as a blocking effect of polysynaptic reflexes[4]. On account of these properties, an antistrychnine action can be expected for the kavapyrones. For one of them, dihydromethysticin (DHM), such an effect was described earlier[5,6].

Methods. In mice, poisoned with various doses of strychnine, the following 5,6-hydrogenated pyrones, kavain (K), dihydrokavain (DHK), methysticin (M), and again dihydromethysticin, were tested in comparison with mephenesin and phenobarbital. The anticonvulsive and the antilethal effect were evaluated. For each of these effects, the $ED_{50} \pm s_{\bar{x}}$ was calculated by the method of MILLER and TAINTER using 10–30 animals for each dose. Male albino mice (weight 20–27 g) were poisoned with strychnine doses from 2 mg/kg (the minimum certainly effective lethal dose) up to 60 mg/kg injected s.c. After the injection of strychnine, the animals were set in separate cages. The compounds tested were injected i.p., the pyrones and phenobarbital 30 min before, mephenesin only 1 min before strychnine. The pyrones were dissolved in peanut oil because of low solubility in water.

Results. All the kavapyrones showed a marked antagonistic effect upon the convulsant and lethal action of strychnine. The mean dose of the compounds tested which antagonize the lethal effect of 2, 3 and 5 mg/kg strychnine are summarized in the Table. M proved to be the most potent compound of all pyrones tested. The ED_{50} of M against 2 mg/kg strychnine amounts to approximately $^1/_{33}$ of the LD_{50} of this drug, against 5 mg/kg strychnine to $^1/_7$ of the LD_{50}. Pretreatment of the animals with the maximum effective dose of M (300 mg/kg) increases the LD_{50} of strychnine 5–6 times. M even protects some animals against the lethal effect of 10 mg/kg strychnine, a dose 5 times higher than the minimum certainly effective lethal dose of the alkaloid. Except for DHM, the other pyrones act in the same range as M, as far as the maximum effective doses and the absolute limits of activity against strychnine are concerned. The protective effect of K and DHK becomes apparent only with higher doses, close to the intrinsic toxic activity of these drugs. DHM acts against strychnine poisoning of 2 mg/kg in the same range as M, but this drug has the lowest absolute limit of activity. Except for DHM, 2–5 mg/kg of strychnine are antagonized by pyrone doses which otherwise influence only very little the behaviour of the animals. The ED_{50} against these strychnine doses are lower than those producing loss of righting reflex or paralysis.

The pyrones have a higher protective activity against strychnine poisoning than mephenesin, which acts only against 2 mg/kg strychnine. The ED_{50} amounts to 40% of the LD_{50} of the latter compound. This dose of mephenesin otherwise provokes complete paralysis of the animals.

[1] R. HÄNSEL and H. RIMPLER, Z. analyt. Chem. *207*, 270 (1965).
[2] H. J. MEYER, Arch. int. Pharmacodyn. *138*, 505 (1962).
[3] R. KRETZSCHMAR and H. J. MEYER, Arch. int. Pharmacodyn. *177*, 261 (1969).
[4] H. J. MEYER and R. KRETZSCHMAR, Klin. Wschr. *44*, 902 (1966).
[5] F. KELLER, R. E. WILLIAMS, M. J. TOEKES, and G. E. CRONHEIM, J. nederl. pharm. Chem. *1*, 95 (1959).
[6] H. J. MEYER, Arch. int. Pharmacodyn. *150*, 118 (1964).

Antistrychnine potency, LD_{50}, and ED_{50} of loss of righting reflex in mice of pyrone compounds and mephenesin and phenobarbital

Drug	LD_{50} ($\bar{x} \pm s_{\bar{x}}$) mg/kg i.p.	ED_{50} loss of righting reflex ($\bar{x} \pm s_{\bar{x}}$) mg/kg i.p.	Antilethal ED_{50} against strychnine ($\bar{x} \pm s_{\bar{x}}$) mg/kg i.p. Doses of strychnine, mg/kg s.c. 2	3	5	Maximal effective doses against strychnine mg/kg i.p.	LD_{50} of strychnine after pretreatment with maximal effective doses mg/kg s.c.[a]	Absolute limit of antagonistic activity at mg/kg strychnine
DHK	490 ± 15.2 ($n = 80$)	325 ± 18.2 ($n = 30$)	122 ± 10.0 ($n = 40$)	175 ± 20.9 ($n = 40$)	225 ± 25.3 ($n = 30$)	300	6.0 ± 0.48 ($n = 50$)	12
K	420 ± 14.7 ($n = 80$)	252 ± 10.2 ($n = 25$)	76 ± 6.4 ($n = 50$)	98 ± 6.8 ($n = 50$)	162 ± 13.4 ($n = 30$)	200	6.8 ± 0.39 ($n = 50$)	12
DHM	420 ± 20.0 ($n = 80$)	225 ± 12.7 ($n = 25$)	18 ± 1.6 ($n = 50$)	59 ± 3.2 ($n = 50$)	–	100	3.8 ± 0.10 ($n = 30$)	5
M	530 ± 29.9 ($n = 60$)	300 ± 27.5 ($n = 20$)	16 ± 1.2 ($n = 50$)	31 ± 3.4 ($n = 40$)	77 ± 9.9 ($n = 40$)	300	7.3 ± 0.45 ($n = 60$)	12
Mephenesin	520 ± 10.3 ($n = 50$)	173 ± 17.4 ($n = 25$)	215 ± 29.8	–	–	300	2.4 ± 0.14 ($n = 30$)	4
Phenobarbital-Na	260 ± 9.8 ($n = 50$)	135 ± 10.1 ($n = 25$)	34 ± 3.6 ($n = 30$)	57 ± 2.6 ($n = 40$)	83 ± 7.3 ($n = 40$)	200	43.0 ± 4.30 ($n = 50$)	60

[a] LD_{50} of strychnine without pretreatment = 1.45 ± 0.05 mg/kg.

Phenobarbital protects against extreme strychnine doses, where highest amounts of pyrones are ineffective. But the maximum effective dose of phenobarbital causes complete anaesthesia and amounts to 75–80% of the LD_{50}. Non-anaesthetic or non-ataxic doses of phenobarbital protect only against 2–3 mg/kg strychnine.

The superiority of the kavapyrones over mephenesin is also manifested in their ability to influence the seizure pattern of strychnine. In this regard the kavapyrones also differ clearly from phenobarbital. Low doses of pyrones up to 150 mg/kg M or DHM and 200 mg/kg K or DHK completely prevent convulsions due to strychnine up to 4–5 mg/kg. The animals are totally unimpaired by the strychnine injection, if they are pretreated with these pyrone doses; in particular they have a normal motor activity and take food. Higher doses of pyrones modify the convulsions due to strychnine producing a characteristic seizure syndrome with long periods of generalized violent clonic convulsions in remarkably brief intervals and with high frequency (15–17 c/sec). The action of higher pyrone doses against strychnine is not specific for the pyrones. Mephenesin and also phenobarbital show this seizure syndrome with strychnine in the total dose range. This phenomenon was earlier described for other barbiturates like nembutal by S. LOEWE[7].

Discussion. The effect of low doses of pyrones, which neither block polysynaptic reflexes nor evoke a distinct muscle relaxation but completely antagonize strychnine doses up to 5 mg/kg, is remarkable insofar as there are no other compounds up to now which antagonize strychnine poisoning and do not influence at the same dose range the behaviour of the animals. The strychnine antagonistic doses of drugs such as mephenesin, meprobamate[8], benzazoles[9] or benzodiazepines[10] produce at the same time loss of postural and righting reflexes or a marked muscle relaxation. Mephenesin, phenobarbital and other barbiturates also do not totally prevent convulsions due to strychnine. They cause a modification of the classical tetanic strychnine convulsions inducing violent clonic seizures. Hence the effect of low doses of pyrones seems to be approximately specific. The mechanism of this action is yet unknown. Possibly such doses alter the relation of strychnine to the inhibitory transmitter substance at the postsynaptic membrane of motoneurons. On the other hand, high doses of pyrones produce like mephenesin and barbiturates a similar seizure syndrome with strychnine. Probably the pyrones possess a second, non-specific antistrychnine mechanism in doses which block polysynaptic reflexes[11].

Zusammenfassung. Aus dem Kawa-Rhizom isolierte Pyronverbindungen erwiesen sich in Versuchen an Mäusen als stark wirksame Antagonisten der tödlichen Strychninvergiftung.

R. KRETZSCHMAR, H. J. MEYER † and H. J. TESCHENDORF

Pharmakologisches Institut der Universität, D-78 Freiburg i. Br. (Germany), 15 October 1969.

[7] S. LOEWE, Arch. int. Pharmacodyn. *114*, 451 (1958).
[8] F. M. BERGER, J. Pharmac. *112*, 413 (1954).
[9] A. P. ROSZKOWSKI, J. Pharmac. *129*, 75 (1960).
[10] L. H. STERNBACH, L. O. RANDALL, and S. R. GUSTAFSON, in *Psychopharmacological Agents*, (Academic Press, New York and London 1964), vol. 1, p. 168.
[11] The authors are indebted to Fa. Spezialchemie GmbH and Co., München, for providing the pyrone compounds.