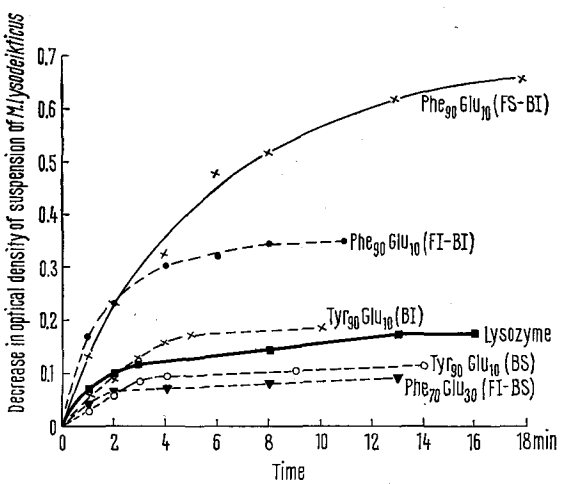
amino-acid in 2 stages. In the first the N-carboxy-anhydride of the hydrophobic amino-acid was in excess, and in the second the ratio was reversed. Subsequent removal of the benzyl groups yielded peptides having carboxyl functions in both hydrophobic and hydrophilic regions. Amino-acid ratios of the active fractions from these copolymerizations, however, revealed that activity was only observable in peptides having an excess of the hydrophobic amino-acid. A random copolymer of Glu and a hydrophobic amino-acid would be expected to have Glu residues and, therefore, carboxylic functions in the inner hydrophobic region as well as the outer surface, which would be in a hydrophilic environment. Copolymers of Phe-Glu, Tyr-Glu and Ala-Glu have, therefore, been synthesized containing different ratios of the 2 amino-acids. Some of these copolymers, indeed, have very substantial lysozyme-like activity (Figure).

For the synthesis of these Glu copolymers, the N-carboxyanhydrides of γ-benzyl-L-glutamate and the second amino-acid in the appropriate ratio were dissolved in dioxane (1 mmol/100 ml) and polymerization initiated with triethylamine (0.005 mmol/100 ml). Deblocking of the precipitated copolymers was achieved by treatment with 2 *N* HBr in AcOH. The Phe-Glu copolymers were separated into formic-acid soluble and insoluble fractions before treatment with HBr, and all copolymers were fractionated into bicarbonate soluble and insoluble materials.



Lytic activity of synthetic Glu copolymers (0.5 mg/ml) and hen egg-white lysozyme (0.05 mg/ml). FI, formic acid insoluble; FS, formic acid soluble; BI, bicarbonate insoluble; BS, bicarbonate soluble.

Lytic activity of the copolymers was assessed by their ability to decrease the turbidity of suspensions of *Micrococcus lysodeikticus* as described earlier[2]. The active copolymers also degraded the cell wall of *M. lysodeikticus* with liberation of reducing sugars. The most active polymer, $Phe_{90}Glu_{10}$[4] and egg-white lysozyme degraded the cell wall of *M. lysodeikticus* to glycopeptides that were indistinguishable on paper electrophoresis (0.1 *M* sodium borate-HCl buffer, pH 6.5; 3.8 volts/cm for 18 h). Paper electrophoresis of 6 *N* HCl hydrolysates of these glycopeptides revealed the same ninhydrin-positive zones. $Phe_{90}Glu_{10}$ has also been found to make amoebic cysts susceptible to emetine hydrochloride in a manner analogous to egg-white lysozyme[5].

It is not possible to get an absolute assessment of the activity of $Phe_{90}Glu_{10}$ as this polymer is insoluble. Assays have, therefore, been carried out with suspensions. Even so, it would appear (Figure) that $Phe_{90}Glu_{10}$ has about $^1/_3$ the activity of egg-white lysozyme and the bicarbonate-soluble $Phe_{70}Glu_{30}$ one-tenth the activity. $Tyr_{90}Glu_{10}$ is far less active than the corresponding Phe-Glu copolymer $Phe_{90}Glu_{10}$. Copolymers $Ala_{90}Glu_{10}$, $Ala_{70}Glu_{30}$, $Ala_{50}Glu_{50}$, $Tyr_{70}Glu_{30}$, $Tyr_{50}Glu_{50}$ and $Phe_{50}Glu_{50}$ are all inactive.

Zusammenfassung. Synthetische Copolymere aus Glutaminsäure und Phenylalanin zeigen Lysozymaktivität. Mit einem Produkt $Phe_{90}Glu_{10}$ wurde ca. ein Drittel der Lysozymaktivität erreicht.

SUSHMA SRIVASTAVA, K. B. MATHUR and M. M. DHAR[6]

Central Drug Research Institute, 173 Lucknow (India), 1 August 1969.

[4] Copolymers designated according to molar percentage of N-carboxyanhydrides used for their synthesis. Actual amino-acid analysis correspond to these values closely.

[5] S. A. IMAM, unpublished data.

[6] To whom enquiries may be made.

Pendulin, a New Biscoclaurine Alkaloid from *Cocculus pendulus* Diels[1]

During a programme for screening plant extracts for biological activities, hypotensive and anticancer activities were seen in the 50% ethanol extract of the leaves and stem of *Cocculus pendulus* (Forsk) Diels (Syn. *C. leaeba* DC)[2]. Follow-up studies located both these activities in the alkaloidal fraction, which, as a result, has been taken up for more detailed studies.

Columbine and palmitine have been found earlier in this plant[3]. More recently coclaurine, menisarine and sinactine have also been located[4].

In the present investigation[5], a new biscoclaurine base, designated pendulin, ($C_{37}H_{40}N_2O_6$) (M^+, 608), mp 192–194°, $[\alpha]_D$ + 265° has been isolated. Pendulin forms a hydrochloride mp 276–278°, a picrate mp 210–212° and a dimenthiodide mp 282–286°. Its IR-spectrum in KBr has absorption bands at 3322, 2857, 1587, 1506, 1458, 1372, 1267, 1221, 1117, 1070, 1020 and 972 cm^{-1} indicating the presence of hydroxyl and ether functions in the molecule and its aromatic nature. Its UV maximum at 284 nm (log ε, 3.84) in ethanol is bathochromically shifted to 306 nm on addition of sodium hydroxide in a manner typical for *bis*(benzyl)isoquinoline bases[6].

[1] Communication No. 1414 from the Central Drug Research Institute.

[2] D. S. BHAKUNI, M. L. DHAR, M. M. DHAR, B. N. DHAWAN and B. N. MEHROTRA, Indian J. exp. Biol., in press.

[3] L. BEAUQUESNE, Bull. Sci. Pharm. *45*, 7 (1938).

[4] A. SINHA, J. Proc. Inst. Chem., India *32*, 250 (1960).

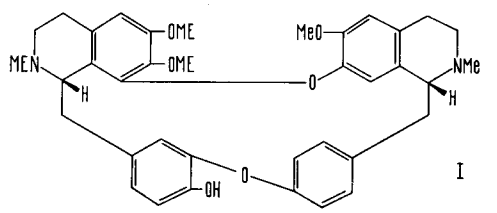
[5] Satisfactory C, H and N analysis of all reported compounds obtained and optical rotations routinely determined in $CHCl_3$ UV-spectra in EtOH, IR-spectra in KBr and 60 Mcs NMR-spectra in $CDCl_3$ with TMS as an internal standard.

[6] A. W. SANGSTER and K. L. STUART, Chem. Rev. *65*, 69 (1965).

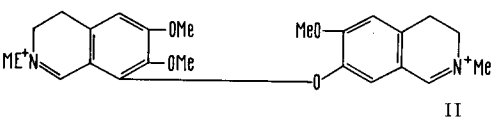
Treatment of pendulin in methanol with ethereal solutions of diazomethane and diazoethane yielded *O*-methylpendulin ($C_{38}H_{42}N_2O_6$) (M^+, 622), mp 150–152°, $[\alpha]_D$ + 210° and *O*-ethylpendulin ($C_{39}H_{44}N_2O_6$) (M^+, 636), mp 144–146°, $[\alpha]_D$ + 218°, respectively. The *O*-methyl derivative formed a hydrochloride mp 272–275° and a picrate mp 251–253°, and the *O*-ethyl derivative, a hydrochloride mp 262–264° (dec.) and a picrate mp 215–218°.

The molecular ion peak (M^+) at m/e 608 in the mass spectrum of pendulin is prominent. Other significant fragments appeared at m/e 607, 416, 396, 395, 381, 364, 349, 198, 175.5, 175, 174. This cracking pattern is characteristic of the oxyacanthine-berbamine type[7] of *bis*(benzyl)isoquinoline alkaloids. The characteristic double charged ion (II) m/e 198, which is the base peak in the spectrum and is accompanied by an isotopic peak at m/e 198.5, eliminates a methoxyl and a methyl radical to give an ion at m/e 175. The key ion at m/e 396, which looses an hydrogen atom to give an ion at m/e 395, is an ion always found in the mass spectra of the oxyacanthine-berbamine type of alkaloids[7], and its presence in the spectrum of pendulin is evidence that pendulin is isomeric with berbamine and oxyacanthine bases. From the fragmentation pattern, it also follows that all 3 methoxy groups present in the pendulin molecule are located in the tetrahydroisoquinoline moieties and the hydroxyl function in either of the 2 benzylic halves.

The NMR-spectrum of pendulin confirmed the presence of 40 protons in this molecule and the pattern of the spectrum was that of *bis*(benzyl)isoquinoline alkaloids[8]. The 2 N-Me functions gave rise to singlets at τ 7.38 and 7.68. Signals for 2 shielded *O*-Me groups appeared together as a singlet at τ 6.79 and the third *O*-Me group resonance was in the normal position at τ 6.25. 10 protons were responsible for the signals in the aromatic region of the spectrum. A shielded proton signal appeared as a singlet at τ 3.95 and a 2-proton singlet was present at τ 3.21. The remaining 7 protons (coupled *ortho*, *para* and *meta*) gave rise to multiplets between τ 2.60–3.80. 4 benzylic, 8-ring methylene and 2-ring methine proton signals were at τ 6.10, 7.12 and 6.58, respectively.



I



II

The NMR-spectra of *O*-methylpendulin and *O*-ethylpendulin were better resolved. The spectrum of *O*-methylpendulin had 4 *O*-Me signals at τ6. 02, 6.20, 6.62 and 6.72. A shielded aromatic proton was coupled with a *para* proton and appeared as a doublet at τ 3.94 (J, 1.5 cps). The 2 aromatic proton singlet at τ 3.08 was somewhat resolved. The other features of this spectrum were similar to those of the spectrum of pendulin.

In the NMR-spectrum of *O*-ethylpendulin, 3 *O*-Me signals were at τ 6.20, 6.63 and 6.78 and the *O*-Et triplet and quartet at τ 8.52 (J, 7 cps) and τ 5.82 (J, 7 cps), respectively. Other features of this spectrum were the same as in the spectrum of *O*-methylpendulin.

Bick et al.[8] have correlated chemical shifts of methoxyl functions with the stereochemistry of *bis*coclaurine bases. When the 2 coclaurine moieties of these bases are paired (+ −) or (− +), the 6′-methoxyl resonance has a chemical shift of τ 6.4, whereas with (+ +) or (− −) paired structures, the chemical shift is near τ 6.65. In *O*-methylpendulin and *O*-ethylpendulin the 6′-methoxyl function resonates at τ 6.62 and 6.63 respectively and therefore, suggests that pendulin exists in *Cocculus pendulus* in either (+ +) or (− −) forms.

The fact that pendulin exists in the (+ +) forms was confirmed by sodium and liquid ammonia reduction of *O*-ethylpendulin. A non-phenolic and a phenolic compound were isolated and found identical with (+) *O*-ethylarmepavin[9] and (+) N-methylcoclaurine[10], respectively. A *bis*coclaurine base pycnamine has recently been isolated from the roots of *Pycnarrhena manillensis*[11] and shown to exist in the (− −) form. Pendulin and pycnamine are, therefore, most probably optical isomers.

Zusammenfassung. Ein Biscoclaurin Alkaloid, Pendulin ($C_{37}H_{40}N_2O_6$), mp 192–194°, $[\alpha]_D$ + 265°, wurde aus Blättern und Stamm von *Cocculus pendulus* Diels isoliert und seine Struktur (I) inklusive der sterischen Anordnung an beiden Asymetriezentren aufgeklärt.

N. C. Gupta, D. S. Bhakuni and M. M. Dhar

Central Drug Research Institute, P.B. 173, Lucknow (India), 30 July 1969.

[7] D. C. De Jongh, S. R. Shrader and M. P. Cava, J. Am. chem. Soc. *88*, 1052 (1966). – M. Tomita, T. Kikuchi, T. Fujitani, A. Kato, H. Furukawa, Y. Aoyagi, M. Kitano and T. Ibuka, Tetrahedron Letters (*1966*), 857. – J. Baldas, Q. N. Porter, I. R. C. Bick and M. J. Vernengo, Tetrahedron Letters (*1966*), 2059.

[8] I. R. C. Bick, J. Harley-Mason, N. Sheppard and M. J. Vernengo, J. chem. Soc. (*1961*), 1896.

[9] T. Tomimatsu, J. pharm. Soc., Japan *79*, 1386 (1959). – E. Fujita and T. Tomimatsu, *79*, 1260 (1959).

[10] M. Tomita and Y. Kondo, J. pharm. Soc., Japan *77*, 1019 (1957). – H. Yamaguchi, *78*, 678 (1958).

[11] G. Aquilarsantos and C. Shäfer, Arch. Pharm. *293*, 785 (1960).

Slow Spontaneous Signals from Brain Tissue Culture

Spontaneous signals have been reported from in vitro brain preparations of most classes of animals[1]. This paper describes spontaneous slow signals from 14-day-old chick embryo telencephalic explants. These may arise in dendrites or in glial cells secondary to neuronal activity.

Material and method. The chamber described previously[2] had a second 90 μ platinum (gross reference) electrode. A thin 1–2 mm diameter slice of right posterior pole of 14-day-old chick embryo telencephalon was placed on the bare tip of the gross recording electrode in the angle be-