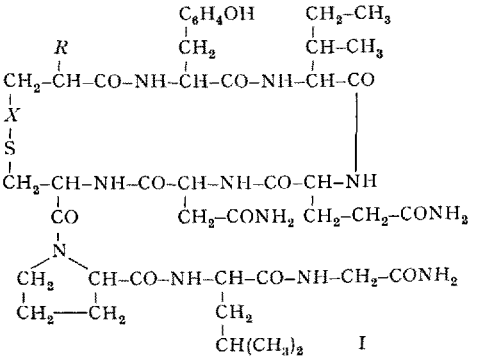
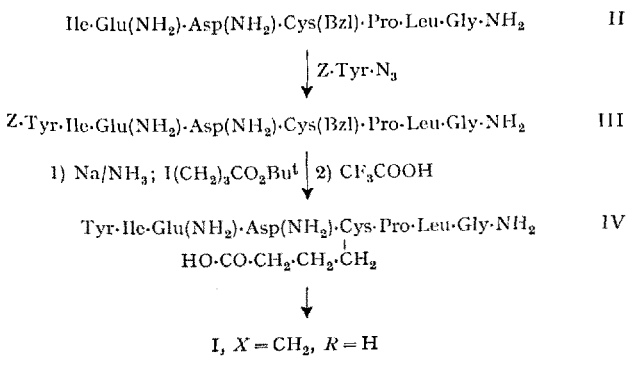
IV was isolated in an analytically pure state as the dihydrate, m.p. 145–147°, by elution from a column of Dowex 1 with a decreasing pH gradient (pyridine-acetic acid) at about pH 5.5. The peptide was cyclized by treatment with N-ethyl-5-phenylisoxazolium-3′-sulfonate[18] in dimethylformamide under high-dilution conditions[19]. The cyclic product (I, $X = CH_2$, $R = H$), which may be named as the lactam of tyrosyl-isoleucyl-glutaminyl-asparaginyl-S-(3-carboxypropyl)cysteinyl-prolyl-leucyl-glycine amide, was isolated after deionization on ion exchange columns as an electrophoretically and chromatographically homogeneous, neutral, ninhydrin-negative material giving a positive Pauly reaction and iodoplatinate(IV) reaction for sulfur. Analysis of the lyophilized product for nitrogen indicated a peptide content of 80%. Amino acid analysis[20] showed the presence of all the expected amino acids (including S-(3-carboxypropyl)cysteine) in equimolecular amounts, except for tyrosine, for which low values were found; we believe this to be due to decomposition rather than inhomogeneity of the product.



I



II

III

IV

When assayed on the rat uterus *in vitro* under standard conditions[21], the analogue had an activity corresponding to about 60 IU/mg; the avian depressor activity[22] was about 25 IU/mg, and the antidiuretic activity in the hydrated alcohol-anaesthetized rat[23] about 1 IU/mg. These results[24] show that at any rate these biological effects do not critically require the presence of the disulfide bond, and preclude molecular mechanisms based on the reactivity of such a bond.

Zusammenfassung. Die Synthese eines Analogen des Deamino-oxytocins, in dem ein Schwefelatom der Disulfidbrücke durch eine Methylengruppe ersetzt ist (I, $X = CH_2$, $R = H$), wird beschrieben. Die Verbindung hat oxytocinähnliche biologische Wirkungen.

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Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Science, Prague (Czechoslovakia), July 13, 1964.

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On the Expansion-Contraction Rhythm of the Sea Anemone, *Actinia equina* L.[1]

The expansion-contraction rhythms (ECR) shown by many Anthozoa have generally been interpreted as daily rhythms. However, an ECR correlated with the tide was also described[2] in *Actinia equina* L., but apparently this species also has a daily ECR[3]. According to Bohn[4], both rhythms are maintained, in the laboratory, under conditions of constant light and constant water level. This fact has been doubted by other authors[5–7].

We have studied specimens of *A. equina* from the intertidal zone of the Tyrrhenian coast. We have collected only specimens which were under the same conditions of illumination and at the same level. They were kept in the laboratory at a temperature of 20°C ± 0.5° and their activity was checked every 30 min. The observations during the dark periods were done using an infrared source and detector (sniper-scope).

The animals were divided into 6 groups: (1) *Continuous immersion, nictemeral rhythm of illumination.* Sea anemones are expanded in the dark and closed in the light. (2) *Continuous immersion, nictemeral illumination and moonlight.* The behaviour of the animals is similar to that of group 1. Moonlight does not seem to have any influence.

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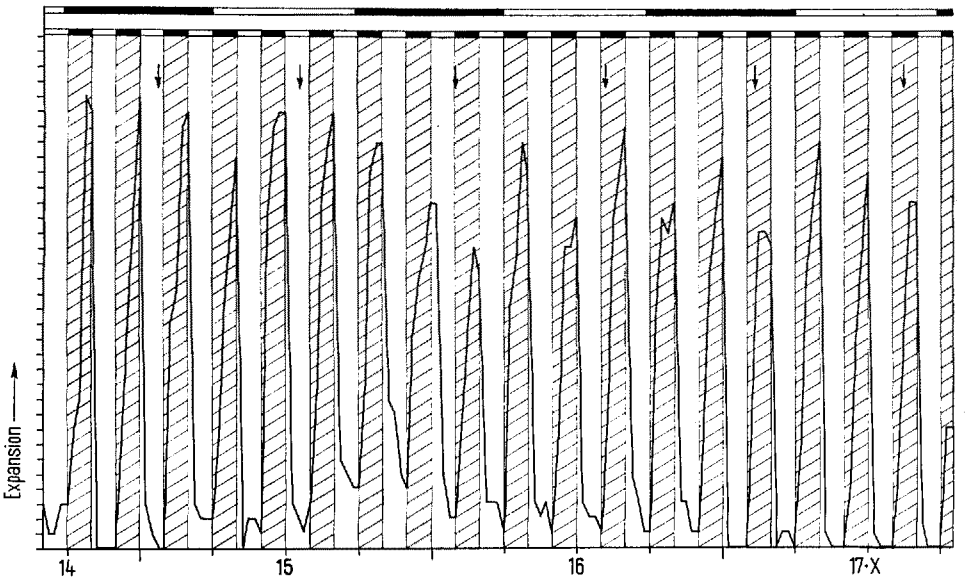

Fig. 1. Cumulative data showing the variation of the state of expansion of 10 specimens of *Actinia equina* L. in 3 successive days; 2:2 h light-dark conditions. Dark periods shaded. At the top, natural day and night. The arrows show the time of low tide on the coast. Abscissa: observation days. Ordinate: degree of expansion expressed in arbitrary units.

(3) *Tidal rhythm, continuous illumination of weak intensity.* The animals are expanded when immersed and closed when exposed. (4) *Tidal rhythm and nictemeral illumination.* The actiniae are fully expanded in the night at high water. (5) *Continuous immersion; weak continuous illumination.* The behaviour shows strong individual differences. Although the animals alternate with phases of expansion and contraction it does not seem possible to individualize any tidal or cyrcadian periodicity. (6) *Continuous immersion. 2 h of light and 2 h of darkness, alternately.* The ECR is adapted to the illumination conditions (Figure). The sea anemones follow the 4 h cycle, although they alternate with periods of minor or major reactivity.

We conclude that, at least for the Mediterranean population of *A. equina*, the cyrcadian and tidal rhythms do not continue in the laboratory. The behaviour of the animals seems to be directly controlled by external factors.

Riassunto. È stato studiato il ritmo di espansione-contrazione di esemplari di *Actinia equina* L. della zona intercotidale del Mar Tirreno, sottoposti a condizioni differenti di illuminazione e di livello d'acqua. Gli autori ritengono che il ritmo sia direttamente dipendente dall'illuminazione ambientale e dallo stato di copertura o scopertura conseguente alle maree. Una luce di intensità pari a quella lunare non esercita alcuna influenza sul ritmo.

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A New Finding about the Structure-Activity Relationship of Cardiotonic Steroids: Lack of Cardiotonic Action in 15α-Hydroxydigitoxigenin[1]

It is now fairly well established that there are certain structural features essential for the cardiotonic activity of cardenolides and bufadienolides. In most of these compounds, the essential features are: *cis*-fusion of the C and D rings, hydroxyl groups in positions 3β and 14β, and a five- or six-membered lactone ring having β-configuration at C_{17}[2].

Quite unexpectedly, however, the authors found that 15α-hydroxydigitoxigenin (15α-OH D-genin) did not show any cardiotonic activity, in spite of the fact that it retains all these essential features intact and just has another hydroxyl group at 15α position[3,4]. In this paper, the effect of this compound upon the heart contractile force will be described in comparison with that of digitoxigenin (D-genin).

Stock solutions of D-genin and 15α-OH D-genin were prepared, dissolving each compound in 70% ethanol in concentration of 1 mg/ml. Just before use, these stock solutions were diluted with Ringer's solution to desired concentrations in frog experiments, and with physiological saline to an appropriate volume in dog experiments.

(1) *Isolated frog's heart (Straub's preparation).* Impairment of the heart contractile force was induced by re-

[1] A part of the expense of this study was supported by a grant from Hoansha Research Fund.

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