

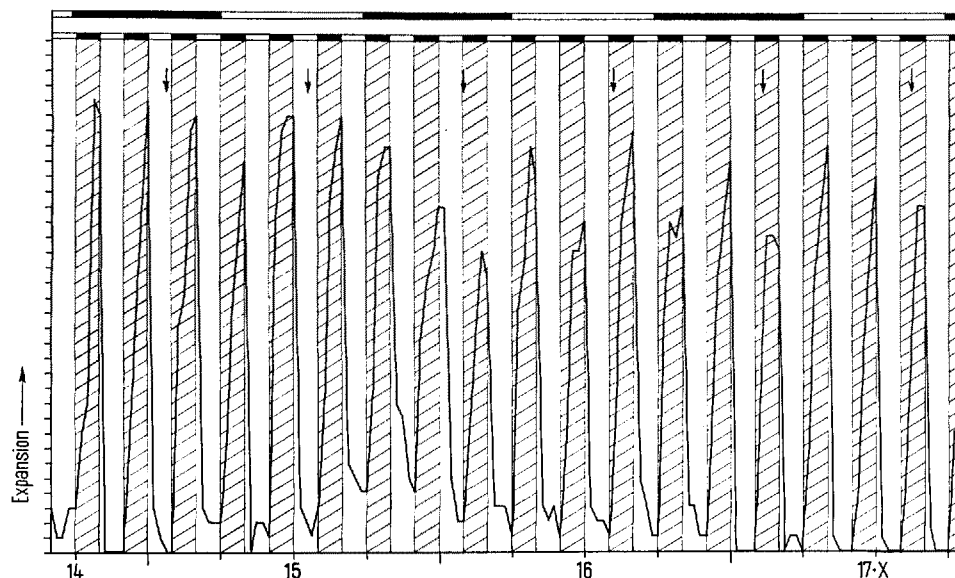


Fig. 1. Cumulative data showing the variation of the state of expansion of 10 specimens of *Actinia equina* L. in 3 successive days; 2:2 h light-dark conditions. Dark periods shaded. At the top, natural day and night. The arrows show the time of low tide on the coast. Abscissa: observation days. Ordinate: 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 circadian 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 circadian 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 15 α -Hydroxydigitoxigenin¹

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 3 β and 14 β , and a five- or six-membered lactone ring having β -configuration at C₁₇².

Quite unexpectedly, however, the authors found that 15 α -hydroxydigitoxigenin (15 α -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 15 α 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 15 α -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.

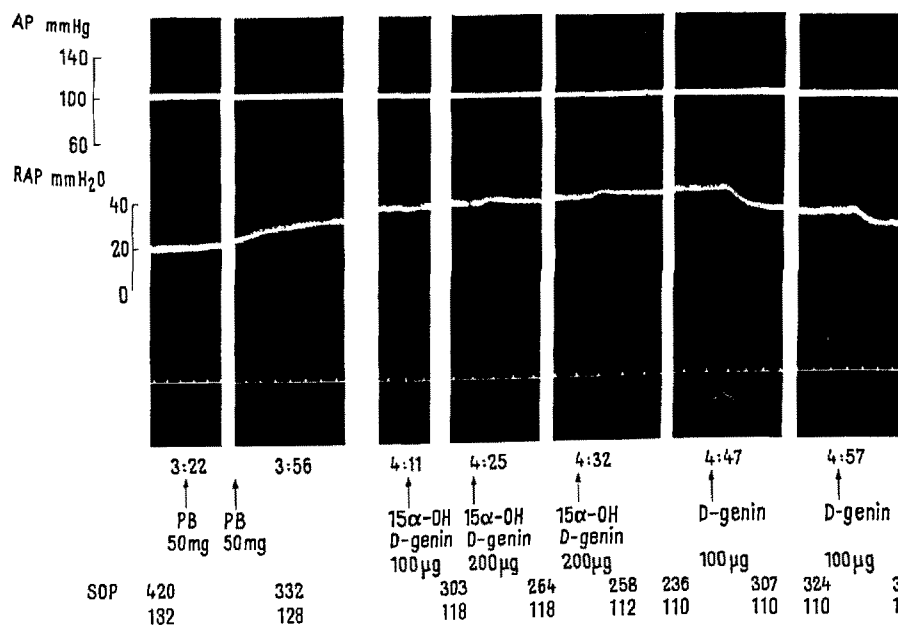
(1) *Isolated frog's heart (Straub's preparation).* Impairment of the heart contractile force was induced by re-

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

² CH. TAMM, Proc. 1st Internat. Pharmacol. Meeting, vol. 3 (Ed. by W. WILBRANDT, Pergamon Press, 1963).

³ M. OKADA and M. HASUNUMA, Proc. 82nd Annual Meeting of the Pharmaceutical Society of Japan (1962), p. 219.

⁴ H. ISHII, T. TOZYO, and D. SATO, Proc. 82nd Annual Meeting of the Pharmaceutical Society of Japan (1962), p. 218; Chemical and Pharmaceutical Bulletin 11, 576 (1963).



Effect of 15 α -hydroxydigitoxigenin and digitoxigenin on the dog heart-lung preparation. The tracings are from top to bottom: arterial pressure (AP), right arterial pressure (RAP), systemic output recorded with a Weese stromuhr. PB = pentobarbital sodium, 15 α -OH D-genin = 15 α -hydroxydigitoxigenin, D-genin = digitoxigenin, SOP = systemic output in ml per min, HR = heart rate per min.

ducing the calcium concentration of the medium to half (0.8 mM). Under this condition, administration of 10⁻⁷ (w/v) of D-genin invariably produced an appreciable increase in heart contractile force. A higher concentration of D-genin showed a more remarkable effect and then caused a typical systolic arrest. In contrast, 15 α -OH D-genin, even in a dose of as high as 10⁻⁵, did not show any improvement in the heart contractility.

(2) *Heart-lung preparation of the dog.* In this preparation heart failure was produced by administering pentobarbital sodium. After a new steady state was reached, 15 α -OH D-genin was injected into the rubber tubing leading to the venous cannula. As is shown in the Figure, even a cumulative dose of as high as 500 μ g of 15 α -OH D-genin could not produce any appreciable change in the heart contractile force. Then 100 μ g of D-genin was injected *via* the same route. This caused a marked decrease in right atrial pressure and an increase in systemic output.

The results of these experiments suggest that the hydroxyl group introduced into 15 α position somehow interferes with the stereochemical arrangement in the vicinity of the C and D rings, depriving the cardiotonic activity of the resulting compound⁵.

Zusammenfassung. Es wurde die inotrope Wirkung von 15 α -Hydroxy-Digitoxigenin am isolierten Herzen des Frosches und am Herz-Lungen-Präparat des Hundes untersucht. Abweichend von Digitoxigenin hat das 15 α -Hydroxy-Derivat keinen Einfluss auf die Kontraktionskraft dieser Präparate.

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⁵ 15 α -OH D-genin used in this study was generously supplied by Dr. MASASHI OKADA of the Tokyo Biochemical Institute, Tokyo (Japan).

Canthaxanthin als Sekundär-Carotinoid einiger Grünalgen

In vielen Fällen ändert sich die Pigmentgaritur von Grünalgen bei Nährstoffmangel, besonders bei Fehlen von Stickstoff¹. Die dabei gelegentlich auftretenden Sekundär-Carotinoide sind mehrfach untersucht^{1,2} und bei der Gattung *Chlorella* auf ihre Bedeutung als taxonomisches Merkmal geprüft worden³. Astaxanthin konnte als eines dieser Pigmente eindeutig identifiziert werden¹.

Während Untersuchungen über die Nitratreduktion⁴ und über die Wirkung von Chloramphenicol auf die Farb-

stoffgaritur einiger Grünalgen⁵ standen uns grössere Mengen an N-Mangel-Algen zur Verfügung und boten uns Gelegenheit, weitere Pigmente genauer zu charakterisieren. Bereits DERSCH¹ beschrieb ein auf Grund seiner Absorp-

¹ G. DERSCH, *Flora* (Jena) 149, 566 (1960).

² Z. ŠESTÁK und M. BASLEROVÁ, *Microalgae and Photosynthetic Bacteria* (1963), p. 423.

³ E. KESSLER, W. LANGNER, I. LUDEWIG und H. WIECHMANN, *Microalgae and Photosynthetic Bacteria* (1963), p. 7.

⁴ F.-C. CZYGAN, *Planta* (Berlin) 60, 225 (1963).

⁵ F.-C. CZYGAN, *Arch. Mikrobiol.* 47, 251 (1964).