Comparison of the activities in the palmar skin conductivity (PSCR) and antipentylenetetrazole (APIS) tests

Drugs	Activities		APIS A
	APIS A	PSCR A	PSCR A
Clonazepam (cmc)	2695 (2558–2855)	351 (325–375)	7.7 (6.8–8.8)
Flunitrazepam (cmc)	3588 (3187–4158)	514 (513–517)	7.0 (6.5–8.1)
Bromazepam (cmc)	671 [a]	316 (281–348)	2.1
Lorazepam (cmc)	1070 (1048–1101)	664 (623–738)	1.6 (1.4–1.8)
Flurazepam (cmc)	518 [a]	325 (299–351)	1.6
Demoxepam (cmc)	451 (441–463)	286 (265–306)	1.6 (1.4–1.7)
Chlorazepate (cmc)	432 (428–437)	287 (256–318)	1.5 (1.3–1.7)
Tetrazepam (cmc)	388 (374–410)	261 (224–298)	1.5 (1.3–1.8)
Medazepam (sal)	399 (391–409)	290 (263–317)	1.4 (1.2–1.5)
Nitrazepam (cmc)	813 (775–863)	598 (580–628)	1.4 (1.2–1.5)
Oxazepam (cmc)	441 (419–477)	371 (348–392)	1.2 (1.1–1.4)
Diazepam (cmc)	620 (569–724)	542 (528–562)	1.1 (1.0–1.4)
Chlordiazepoxide (cmc)	373 (357–398)	387 (367–405)	1.0 (0.9–1.1)
Phenobarbital Na (sal)	325 (322–329)	343 (324–361)	0.9 (0.89–1.01)
Meprobamate (sal)	283 (277–290)	300 (273–326)	0.9 (0.8–1.1)

Figures in brackets = confidence limits p 0.05.
sal, in Na Cl solution; cmc, in aqueous solution of 0.5% carboxymethylcellulose. [a] Approximate value.

death in 50% of the mice (APIS PD 50 mg/kg), the dose producing a 50% inhibition of PSCR (PSCR ID 50 mg/kg), the corresponding activities:

$$\frac{10^3}{\log (\text{APIS PD } 50 \times 100)} (= \text{APIS A}) \text{ and } \frac{10^3}{\log (\text{PSCR ID } 50 \times 100)}$$

(= PSCR A) and the ratio $\frac{\text{APIS A}}{\text{PSCR A}}$.

The results are given in the Table.

Discussion. Since the various compounds showed greatly differing potencies in the two tests, APIS and PSCR-tests are likely to indicate two different pharmacological actions. The mechanism of the activity of benzodiazepines in APIS-test probably involves an antagonism of the pentylenetetrazole-induced decrease of the presynaptic inhibition in the reticular formation[4]. In the PSCR-test, the effect of benzodiazepines is likely to be the consequence of activity on the ascending reticular formation[5], on autonomic centres and pathways and also on visual pathways[6] as PSCR is elicited by a photostimulus. It is therefore no surprise to find that drugs exhibit different activities in these two tests. So specifically 'anticonvulsant' benzodiazepines (flunitrazepam and clonazepam) reveal an activity ratio APIS A/PSCR A of 7. However, 'sedative' benzodiazepines present an activity ratio which is either around 1, like meprobamate and phenobarbital, or close to 1.5.

Résumé. La comparaison des activités de 13 benzodiazépines sur les tests de réponse de conductivité cutanée palmaire et antipentétrazole permet de différencier par leurs rapports d'activité les benzodiazépines «sédatives» (1,0 à 1,6) et «antiépileptiques» (7).

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Cardiotonic Activities of 3,5-Seco-4-Nor-Cardenolides in *Rana nigromaculata*

In the course of our studies[1–5] on the structure-activity relationship of the cardenolide, several 3,5-seco-4-nor-cardenolides were prepared from digitoxigenin (I), and their cardiotonic activities were tested by using the isolated frog heart (Straub's preparation). In this preliminary report, we describe the cardiotonic activities of the following 4 compounds[6] in comparison with that of digitoxigenin: 14-hydroxy-3,5-seco-4-nor-5-oxo-14β-card-20(22)-enolid-3-oic acid (IIa), its methyl ester (IIb), 5β,14-dihydroxy-3,5-seco-4-nor-14β-card-20(22)-enolid-3-ol (III), 5α,14-dihydroxy-3,5-seco-4-nor-14β-card-20(22)-enolid-3-ol (IV).

The method of assay is the same as described in the previous papers[1–5]. Frogs, *Rana nigromaculata*, were used. The Straub's cannula contained 2 ml of Ringer's solution, the composition of which was: NaCl, 111 mM; KCl, 2.7 mM; $CaCl_2$, 1.8 mM; $NaHCO_3$, 15 mM, and glucose, 2.7 mM. It was aerated with 95% O_2 + 5% CO_2. The contraction of the heart was recorded with isotonic lever on smoked drums. The heart was first made hypodynamic by reducing the concentration of calcium to 0.6 mM, $^1/_3$ of the normal, and then the effect of one of the compounds was tested in the following way.

Stock solutions of the 5 compounds were prepared with 95% ethanol in concentration of 1 mg/ml. Before experiment, these stock solutions were diluted with 0.6% saline to the desired concentrations. Starting from a subthreshold dose, a small amount (20-140 µl) of a diluted solution was added to the cannula every 15–25 min, so that a stepwise increase in the cumulative concentration of the test

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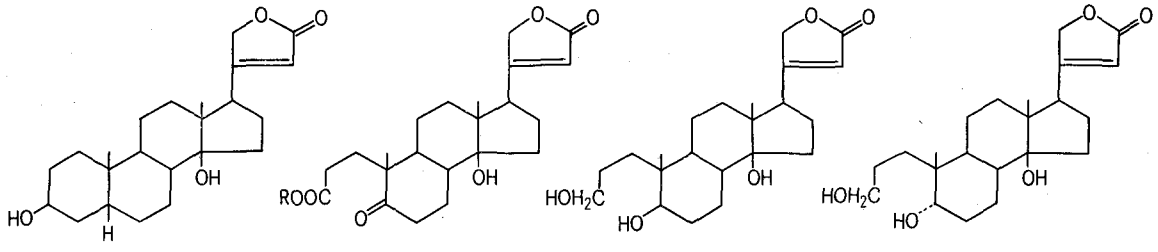
compound was achieved, until the heart went into systolic contracture. The way of increasing the cumulative concentration was: 10^{-n}, 3×10^{-n}, $10^{-(n-1)}$, $\cdots$. Whenever the height of contraction reached a plateau, the next addition was made. The relative potencies were obtained on the basis of the concentration of each compound in which systolic contracture of the heart was brought about (C-contr.).

Each compound was tested on 4 preparations. The experiments were carried out at room temperature of 18–22 °C in November 1974. The results are summarized in the Table.

With digitoxigenin (I), the hearts of all 4 frogs went into systolic contracture at the concentration of 3×10^{-7} g/ml. This is the same concentration as that obtained in summer frogs. C-contr. of IIb was 3×10^{-6} g/ml in 2 hearts, and 10^{-5} g/ml in the other two. C-contr. of III was 3×10^{-5} g/ml in all 4 frogs. Compounds IIa and IV failed to cause systolic contracture at 3×10^{-5} g/ml, which was the highest concentration tested. With compound IV, however, some positive inotropic action was observed at concentrations of 10^{-5} and 3×10^{-5} g/ml, while IIa did not show any effect at these concentrations. Thus the order of potency should be I > IIb > III > IV > IIa.



I IIa: R=H IIb: R=CH_3 III IV

The whole pattern of the response of the Straub's preparation to compounds IIb and III was the same as that of its response to digitoxigenin, and a typical systolic contracture was the final phenomenon. In addition, it was observed in preliminary experiments that the potassium-induced contracture in the frog ventricular muscle strip was markedly potentiated by IIb and III. Therefore, the cardiotonic action of these compounds is certainly of the same nature as that of digitoxigenin or other cardenolides.

It was previously demonstrated that the oxygen function at C–3 is not an indispensable requirement for cardiotonic activity, since 3-deoxydigitoxigenin proved to be almost equipotent with digitoxigenin when tested for its effect on the isolated heart[3] or for its inhibitory action on Na^+, K^+-ATPase[7]. Then, to see if the steroidal skeleton of the cardenolide is an essential structural requirement for cardiotonic activity, 3,5-seco-4-nor-cardenolides were prepared by cleaving the A-ring of digitoxigenin and their effects on isolated cardiac muscle were tested. The present result demonstrates that the perhydrocyclopentanophenanthrene nucleus is not an indispensable requirement for the cardiotonic activity, because 14-hydroxy-3,5-seco-4-nor-5-oxo-14β-card-20(22)-enolid-3-oic acid methyl ester (IIb) and 5β,14-dihydroxy-3,5-seco-4-nor-14β-card-20-(22)-enolid-3-ol (III) produced a definite cardiotonic action in the Straub's preparation. Preparation and pharmacological test of some other 3,5-seco-4-norcardenolides are now in progress in our laboratories and the results will be reported elsewhere, with special reference to the structure-activity relationship[8].

Final concentrations and the relative potencies of the compounds used

Compound	I	IIa	IIb	III	IV
C-contr. (g/ml)	3×10^{-7}	$>3\times10^{-5}$	3×10^{-6} $\sim10^{-5}$	3×10^{-5}	$>3\times10^{-5}$
RP	1.0	(—)	0.03 $\sim$0.1	0.01	<0.01

C-contr., concentration of the compound tested at which a systolic contracture was brought about; RP, relative potency.

Zusammenfassung. Die cardiotonische Aktivität von 4 neuen, aus Digitoxigenin hergestellten 3,5-Seco-4-norcardenoliden auf das isolierte Froschherz wurde geprüft. Die Ergebnisse zeigen, dass für die positive inotrope Wirkung eines Cardenolids das intakte Steroidgerüst nicht notwendig ist.

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