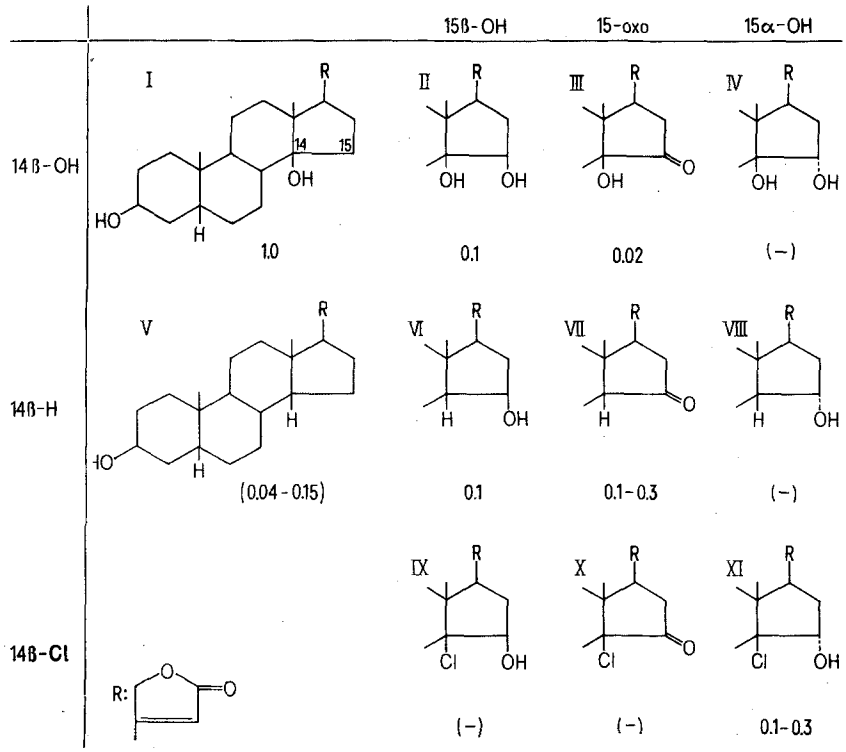

Structural formulae and relative potencies of digitoxigenin (I) and 10 derivatives (II–XI).

concentration was: 10^{-n}, 3×10^{-n}, $10^{-(n-1)}$, $3\times10^{-(n-1)}$, $10^{-(n-2)}$. The relative potencies were obtained on the basis of the concentration of each compound in which systolic contracture of the heart was brought about. As discussed elsewhere[4], this way of comparison provides reasonable values of relative potency. The experiments were carried out at room temperature of 20–26 °C.

The results are summarized in the Formulae. In the 14-deoxy-14βH-series (V–VIII), the relationship was similar, as a whole, to that in the 14β-hydroxy series (I–IV), 15α-hydroxy derivative being inactive again. In the 14β-Cl series (IX–XI), however, the results were quite unexpected. 15β-Hydroxy and 15-oxo derivatives were both inactive, while 15α-hydroxy derivative showed the relative potency of 0.1–0.3.

The relative potency of 14-deoxy-14βH-digitoxigenin was estimated as (0.04–0.15), about $^1/_{10}$ of digitoxigenin. (The figures are put in brackets, since the compound was not perfectly pure.) This confirms again the fact that 14β-hydroxyl group is not indispensable for the cardiotonic activity. The quantitative relationship is, however, not the same as our earlier observation that 14-deoxy-14βH-uzarigenin is about one third as active as uzarigenin. At present it is very difficult to draw any simple conclusion regarding the structure-activity relationship at C-14 and C-15[7].

Zusammenfassung. Es wurden die kardiotonischen Wirkungen sechs neuer Derivate von 14-Deoxy-14βH-digitoxigenin und 14-Deoxy-14β-chloro-digitoxigenin auf das isolierte Froschherz untersucht, sowie die Beziehungen zwischen deren chemischen Strukturen und Wirkungen diskutiert.

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Sodium [*o*-[(2,6-dichlorophenyl)-amino]-phenyl]-acetate (GP 45 840), A New Non-Steroidal Anti-Inflammatory Agent[1]

A series of substituted phenylaminophenylacetic acids was synthetized in an attempt to develop a non-steroidal anti-inflammatory compound with potent biological activity. This programme was based on the hypothesis that two structural features are essential to achieve such a pharmacodynamic effect: an acidic function giving a pH of about 6 and two aromatic nuclei whose substitution inhibits coplanarity[2]. Of the various compounds synthesized, GP 45 840, the sodium salt of the [*o*-[(2,6-Dichlorophenyl)-amino]-phenyl] acetic acid proved highly active in the animal test systems most relevant to the pharmacological characterization of such therapeutic agents.

As is shown in Figure 1, GP 45 840 displays a potent, dose-dependent, anti-inflammatory effect in the carrageenin paw-oedema test[3]. The compound already inhibits the formation of oedema when it is administered orally in a dose as low as 1 mg/kg. Since a reduction of more than 50% is rarely attained in this assay, the action elicited by

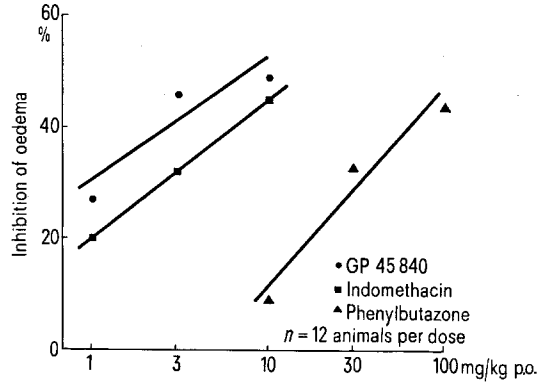

Fig. 1. Effects of GP 45 840, indomethacin and phenylbutazone on rat-paw oedema induced by subplantar injection of 0.05 ml of a 1% (w/v) solution of carrageenin. The compounds were administered 1 h before and the intensity of the oedema was determined gravimetrically 5 h after the injection of carrageenin.

Effects of GP 45 840, indomethacin and phenylbutazone on the incidence of the writhing syndrome induced by i.p. injection of 0.2 ml phenyl-*p*-benzoquinone (0.025% w/v)

Antinociceptive activity, mouse (phenyl-*p*-benzoquinone test)			Acute oral toxicity, mouse
Drug	ED_{50} (mg/kg p.o.) ± S.E.	*n*	LD_{50} (mg/kg p.o.)
GP 45 840	4.3 ± 2	45	ca. 390
Indomethacin	2.7 ± 1.3	45	ca. 50
Phenylbutazone	95 ± 67	45	ca. 1100

The compounds were administered 60 min before induction of the syndrome. The dose that inhibits the writhing frequency by 50% (ED_{50}) was determined according to MILLER and TAINTER.

a dose of 10 mg/kg may be considered a near maximal effect. GP 45 840 is distinctly more active than phenylbutazone and is comparable in potency to indomethacin. However, since the acute oral toxicity of GP 45 840 amounts to about 150 mg/kg in rats, its therapeutic index is greater than that of indomethacin and phenylbutazone. Analogous results have been obtained in other acute models of inflammation, such as kaolin rat-paw oedema[4] and ultraviolet erythema in guinea-pigs[5]. In addition, GP 45 840 retains its full anti-inflammatory activity in adrenalectomized animals. Its anti-inflammatory action is therefore not mediated by activation of the pituitary-adrenal axis.

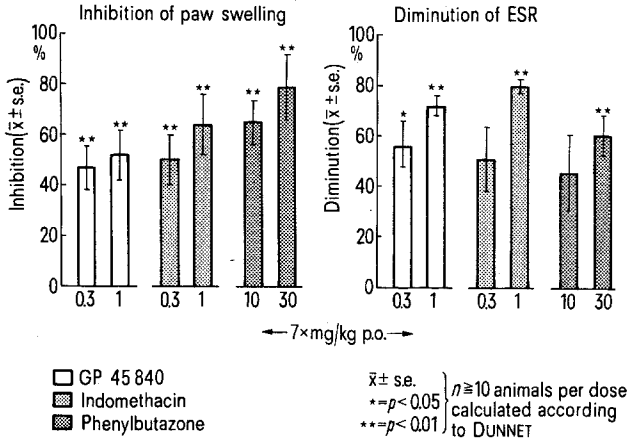

Fig. 2. Effects of GP 45 840, indomethacin and phenylbutazone on paw-swelling and the erythrocyte sedimentation rate (ESR) in the adjuvant arthritis test in rats. The compounds were administered orally for 7 consecutive days, beginning on the 15th day after the injection of complete Freund's adjuvant (1 mg *M. butyricum* in 0.1 ml paraffin oil) into the root of the tail. The swelling of the hind paws was determined volumetrically before and after this treatment, and the ESR measured at the end of the experiment.

GP 45 840 also exerts an anti-inflammatory effect in more chronic types of experimental inflammation, such as the cotton-pellet test[6] or the adjuvant arthritis test in rats[7]. This is exemplified by the results obtained in the established adjuvant arthritis test shown in Figure 2. GP 45 840 brings about a dose-dependent reduction of the arthritic paw-swelling and tends to restore the erythrocyte sedimentation rate to normal. These effects are already seen in response to an oral dose of 0.3 mg/kg daily. The compound is thus comparable in potency to indomethacin, whereas phenylbutazone is distinctly less active.

The antinociceptice effect of GP 45 840 is likewise pronounced. The preparation inhibits the writhing syndrome[8] induced by phenyl-*p*-benzoquinone in mice at low doses (Table). In this test system also, GP 45 840 and indomethacin are virtually equally potent, whereas phenylbutazone is appreciably less effective. However, GP 45 840 has the widest acute therapeutic margin. This is due to its low acute toxicity in mice, which amounts to about 390 mg/kg p.o., when compared with indomethacin, and primarily to its greater potency in the writhing test, when compared with phenylbutazone.

GP 45 840 exerts other interesting additional pharmacodynamic effects. The compound stabilizes platelets, as is demonstrated by its capacity to inhibit aggregation induced in platelet-rich plasma by ADP[9], reduces fever produced by i.m. injection of yeast in rats[10], and suppresses the bronchoconstriction induced by i.v. injections of bradykinin in guinea-pigs[11]. This last-mentioned effect provides indirect evidence that GP 45 840 inhibits

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bradykinin-induced release of prostaglandin-like material[12].

Zusammenfassung. GP 45 840, das Natriumsalz der [*o*-[(2,6-Dichlorphenyl)-amino]-phenyl]-essigsäure, besitzt pharmakodynamisch in verschiedenen Testsystemen am Tier eine ausgeprägte anti-inflammatorische, antinociceptive und antipyretische Aktivität. Das Präparat zeigt eine höhere Wirksamkeit als Phenylbutazon und ist ebenso aktiv wie Indomethacin; es übertrifft in seiner akuten therapeutischen Breite die beiden Vergleichspräparate.

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6 December 1972.

Effect of Spreading Depression on Electrical Activity and Dopamine Turnover in the Striatum of Rats

Epidural application of hyperosmolar KCl solutions on one hemisphere of the rat brain produces depolarization which spreads to the whole cortex[1-4] and the striatum of the ipsilateral side[5] (spreading depression, SD). The SD is associated with an increase in the turnover of brain stem norepinephrine[6] and of striatal dopamine (DA)[7,8]. However, the changes in electrical activity as well as the correlation between biochemical and neurophysiological events during a prolonged SD are not well known. Therefore, in the present paper the effect of epidural application of KCl during 1 h on the electrical activity and the DA turnover in the basal ganglia have been investigated.

Methods. Male albino rats of Wistar origin (Füllinsdorf), weighing 200–250 g, were anaesthetized with Thiogenal®. In a first group of 15 animals, 2 screw electrodes were implanted on each parietal bone 2 and 4 mm, respectively, from the sagittal line and 5 mm anterior to the lambda. Two concentric electrodes (0.3 mm in diameter) were placed in each corpus striatum (coordinates: A = 8.2, L = 3.0, V = +6.0)[9]. An electrolytic lesion made at the end of each experiment facilitated the histological control. All bipolar derivations were connected to a Grass polygraph (model 7B) through a shielded cable attached to an Amphenol strip plug cemented to the skull. A sealed plastic cannula containing a cotton plug moistened with 0.9% NaCl was placed over the dura of the right hemisphere exposed by a skull opening 2 mm anterior and 3 mm lateral to the lambda. A second group of 92 animals in which only the plastic cannula was implanted served for the biochemical assays.

Twenty to 24 h after the operation, the cotton plug in the plastic cannula was replaced by another one impregnated with KCl (25%) or NaCl (20%) which in the first group of rats was again replaced after 1 h by physiological saline. The electrical activity was recorded during the 30 min prior to and 4 h following application of KCl (25%) or NaCl (20%), the rats being kept in a large bell-shaped glass jar under normal laboratory conditions.

In the second group, some of the rats were decapitated 30 or 60 min after epidural application of the concentrated electrolyte solutions, whereas in others KCl (25%) or NaCl (20%) was again replaced after 1 h by 0.9% NaCl. The animals were then sacrificed at various time intervals. Rats killed 20–24 h after the operation without epidural treatment served as controls.

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Effect of epidural application of NaCl or KCl on the content of endogenous homovanillic acid (HVA) in brain of rats

Min after electrolyte application	NaCl, 20%		KCl, 25%	
	L	R	L	R
0	0.092 ± 0.006	0.096 ± 0.005		
30	0.098 ± 0.007	0.100 ± 0.008	0.112 ± 0.003	0.149[a] ± 0.007
60	0.098 ± 0.004	0.106 ± 0.005	0.108 ± 0.005	0.170[a] ± 0.008
120	0.100 ± 0.006	0.109 ± 0.006	0.103 ± 0.003	0.146[a] ± 0.003
180	0.100 ± 0.005	0.100 ± 0.004	0.103 ± 0.004	0.128[a] ± 0.006
240	0.105 ± 0.010	0.112 ± 0.005	0.093 ± 0.008	0.098 ± 0.009

20% NaCl or 25% KCl was applied on the dura of the right cerebral cortex at time 0. Some of the rats were decapitated 30 or 60 min later, whereas in others the concentrated electrolytes were replaced after 60 min by 0.9% NaCl, and sacrifice followed at the time intervals indicated. Operated animals without epidural treatment served as controls (= values for time 0). The concentration of HVA was determined in 2 pooled left (L) or right (R) cerebral hemispheres and is expressed in µg/g wet weight. The values represent means with SEM of all determinations in 2–3 experiments, each performed with 4 rats per group. [a] $p < 0.01$ compared to the corresponding left hemisphere. All the other values of the right hemisphere are not significantly different ($p > 0.05$) from those of the corresponding left hemisphere.