Effect of Simple Phenolic Glycosides on the Elongation of *Avena* First Internodes

Phenolic compounds are considered to day as an individual group of plant growth regulators[1]. The mechanism of their action might be different[2-4]. Phenolic compounds and their derivatives, e.g. glycosides and glucose esters were considered in the past to be waste by-products of plant metabolism. Recently phenolics were found to inhibit of stimulate the oxidative degradation of IAA[5]. Glycosides and glucose esters being the most important bound forms of phenolic compounds in vivo. The enzymes, glycosidases, are supported to be involved in the maintainance of free phenolics pool within the cells. We have reported[6], that β-glycosidase and IAA-oxidase systems are linked through glycosides and their biochemically active aglycones. To demonstrate such a correlation also in vivo we have studied the effect of simple phenolic glycosides on the elongation of *Avena* coleoptile sections in absence of exogenous IAA.

Methods. The plant material was tested with arbutin and gein (eugenol-β-vicianoside; end concentrations 10^{-3} to $10^{-5}M$) in incubation medium according to HENDERSON and NITSCH[7]. Incubation medium contained phosphate buffer pH 5.5, 2% sucrose and 0.1% Tween 80. In control experiments the mentioned solution with and without 1% glucose was used. The initial length of sections was 2 mm. The growth of the sections was measured after 20 h of incubation at 25°C in the dark.

Results and discussion. The results (average of 4 sets of 60 sections each) are shown in the Table. The results were evaluated statistically using the t-test and were demonstrable at P 0,01 level.

Both arbutin and gein in all concentration tested stimulated the elongation of *Avena* coleoptile sections. The glucose in control samples was without any effect which demonstrated that glucose set free from glucosides did not interfere with growth of plat metarial.

These results correspond to the effects of quinol and eugenol on the purified IAA-oxidase described previously[6].

Based on our findings the relationship between IAA-oxidase and β-glycosidase have been found not only in vitro, but also in vivo.

Zusammenfassung. Nachweis, dass phenolische Glykoside Arbutin und Gein das Wachstum der *Avena*-Koleoptilen-Schnitte positiv beeinflussen, was auf eine Beziehung dieser Glycoside zu wachstumregulierenden Mechanismen hinweist.

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The Effect of arbutine and gein on the oat coleoptile growth

Concentration (M)		Growth (%)
Arbutine	10^{-3}	109,7
	10^{-4}	114.5
	10^{-5}	106.9
Gein	10^{-3}	106.7
	10^{-4}	118.3
	10^{-5}	107.2
Control with glucose (1%)		100

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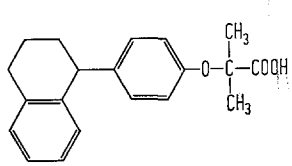
Comparative Studies on the Action of the Optical Antipodes of the Hypolipidaemic Aryloxyalkanoic Acid, CH 13 437, on Liver Enzymes of the Rat

2-Methyl-2-[*p*-(1, 2, 3, 4-tetrahydro-1-naphthyl)-phenoxy]-propionic acid (CH 13437), a compound which belongs to the group of aryloxy-alkanoic acids, is a substance with a strong hypolipidaemic action[1,2]. In experiments with humans, it shows also a distinct lowering of the blood lipids[3-5]. Biochemical investigations of the action on liver metabolism of the rat indicate an inhibition of lipogenesis as the cause of the hypolipidaemic action[6-8].



As shown by the Formula, compound CH 13437 possesses an asymmetric carbon atom in the 1-position of the tetrahydronaphtalene ring. Consequently we were interested in comparing the action of the optical antipodes, and for this purpose we examined in rats those liver enzymes of the carbohydrate and lipid metabolism which in earlier

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experiments with the racemate had shown characteristic activity changes[6], namely pyruvate kinase (EC. 2.7.1.40), citrate synthase (EC 4.1.3.7), citrate cleavage enzyme (EC 4.1.3.8) and malic enzyme (EC 1.1.1.40). Besides these fructose-6-phosphate kinase (EC 2.7 1. 11) and lactate dehydrogenase (EC 1.1.1.27) were investigated. In addition, the relative liver weight, total glycerol and free fatty acids were determined in the serum.

Racemate cleavage was accomplished by using the following method: By means of salt formation with brucine in ethyl acetate solution, at the beginning about 80% of the (+)-CH 13437 was separated. After recrystallizing the brucine salt twice from ethyl acetate solution, the free acid was isolated and recrystallized from cyclohexane solution. Melting point 118 °C, $[\alpha]_D^{20}$ +27° (1% in methanol).

It was more difficult to isolate the pure (−)-form. For this purpose it was necessary to complete separation of the brucine salt of the (+)-acid by adding ether to the mother liquor; in doing so part of the (−)-acid was simultaneously precipitated. The (−)-form of CH 13 437 with a specific rotation of −24° was obtained from the filtrate. Final purification of this product was carried out via the (+)-amphetamine salt (from ethanol solution), and the acid that was isolated from it recrystallized from cyclohexane solution. Melting point 118 °C, $[\alpha]_D^{20}$ −27° (1% in methanol).

A mixture of equal parts of the (+)- and (−)-form melts at 127–128 °C like the original, optically inactive CH 13437[1].

Male Wistar rats at an initial weight of about 250 g received the drug dissolved in polyethylene glycol (molecular weight 400) for 15 days. A daily dose of 10 mg/kg was administered by stomach tube (0.2 ml/100 g body wt.). Feed ('Altromin'-R) and water were freely available. The animals serving as the controls were kept under identical conditions and received only the solvent, polyethylene glycol. On the last day of treatment, 2 h after application, the rats were killed by a blow in the neck and exsanguinated. The livers were removed immediately and homogenized with ice cooling, using the methods described previously[6].

Fructose-6-phosphate kinase, pyruvate kinase and lactate dehydrogenase were determined according to Bücher et al.[9], malic enzyme according to Ochoa[10], citrate cleavage enzyme according to Srere[11] and citrate synthase by the method of Ochoa[12], with reference to the modifications described by Brdiczka et al.[13]. The enzyme activities are calculated in international units (1 IU = 1 µmole substrate exchange per min at 25 °C), related to 1 g liver (fresh wt.). The total glycerol in the serum was determined in accordance with the method of Eggstein and Kreutz[14] and the free fatty acids by the method of Keul et al.[15].

The results of the liver enzyme determinations are compiled in Table I. The first of the two numbers listed, both of which express the change in percents, refers in each case to the (−)-CH 13437 form, and the second one to the (+)-CH 13437 form. Compared to the controls, pyruvate kinase is reduced on average by 45 and 39%, respectively,

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Table I. Comparison of the action of the optical antipodes of CH 13437 on liver enzymes in the rat[a]

Enzyme	Control (mean ± S.D.)	(−)-CH 13437 (mean ± S.D.)	Significance[b] (*P*)	(+)-CH 13437 (mean ± S.D.)	Significance[b] (*P*)
Fructose-6-phosphate kinase	2.44 ± 0.28	2.35 ± 0.64	n.s.	2.08 ± 0.34	<0.05
Pyruvate kinase	25.30 ± 9.95	13.90 ± 4.68	<0.05	15.50 ± 3.68	<0.05
Lactate dehydrogenase	397.6 ± 28.8	579.0 ± 34.9	<0.001	516.6 ± 65.7	<0.001
Citrate synthase	6.10 ± 0.32	6.83 ± 0.67	<0.05	6.66 ± 0.56	<0.05
Citrate cleavage enzyme	1.57 ± 0.44	0.89 ± 0.17	<0.01	0.96 ± 0.31	<0.01
Malic enzyme	1.00 ± 0.20	3.94 ± 1.78	<0.001	2.47 ± 0.59	<0.001

[a] Male Wistar rats (8 animals per group), treated for 15 days with 10 mg/kg/day. Enzyme activities are expressed in IU/g liver. [b] The significance as compared to the controls has been calculated in accordance with Student's *t*-test.

Table II. Comparison of the action of the optical antipodes of CH 13437 on the relative liver weight and blood lipids of the rat[a]

	Control (mean ± S.D.)	(−)-CH 13437 (mean ± S.D.)	Significance[c] (*P*)	(+)-CH 13437 (mean ± S.D.)	Significance[c] (*P*)
Relative liver weight[b]	3.95 ± 0.41	4.11 ± 0.32	n.s.	4.23 ± 0.23	n.s.
Total glycerol[c]	1.93 ± 0.61	1.28 ± 0.27	<0.05	1.24 ± 0.30	<0.05
Free fatty acids[d]	0.27 ± 0.062	0.44 ± 0.132	<0.01	0.37 ± 0.135	<0.01

[a] Male Wistar rats (8 animals per group), treated for 15 days with 10 mg/kg/day. [b] liver weight/body weight × 100. [c] mMol/l. [d] mEqu/l. [e] The significance as compared to the controls has been calculated in accordance with Student's *t*-test.

and citrate cleavage enzyme by 43 and 39% respectively. Lactate dehydrogenase exhibits an activity that is increased by 48 and 30% respectively; in the case of malic enzyme the increase of activity lies by 294 and 147% respectively, and therefore is more pronounced. On the other hand, citrate synthase rises by only 12 and 9% respectively.

In animal experiments the optical antipodes of the compound CH 13437 cause the same alterations in enzyme activities. Neither qualitatively nor quantitatively do they differ in their influence upon liver metabolism. The relative liver weight increases by 4.2% and 7.2% respectively, as compared to the control group (Table II).

In accord with the assumption that the hypolipidaemic action of the drug may be found in a direct influence upon the liver metabolism[1,2], the decrease of triglyceride, measured by the drop in total glycerol, is virtually equal for the antipodes, i.e. 34% for (—)-CH 13437 and 36% for (+)-CH 13437 (Table II).

The increase in free fatty acids in the serum may be connected with inhibition of 3′, 5′-AMP-phosphodiesterase; inhibition of this enzymatic step leads by way of an increase of the 3′, 5′-AMP level to a lipase activation and thus to increased lipolysis[16]. In vitro, 3′, 5′-AMP-phosphodiesterase is inhibited by CH 13437 to approximately the same degree as by theophyllin[17].

The changes in liver enzyme activities, especially reduction of the citrate cleavage enzyme, which are observed after several days of treatment with the drug, lead to inhibition of lipogenesis as the cause of the hypolypidaemic action[6]. In the rat an increase of the smooth, endoplasmatic reticulum of the liver cell, which is also morphologically detectable, and an elevation of the activities of constitutive enzymes are probably the activating primary factors. This results in an induction of NADPH-cytochrome c-reductase and other microsomal enzymes[18]. The increased influx of reduction equivalents via NADPH oxidation induces the malic enzyme. The greater demand for reduction equivalents is initially made available as NADH, as the lactate dehydrogenase activity is increased and makes possible an accelerated dehydrogenation of the lactate transported to the liver. Via an ATP-dependent reaction cycle which proceeds between pyruvate, oxaloacetate and malate, hydrogen may be transferred from NADH to NADPH. Its oxidation finally can be achieved by the microsomal NADPH-cytochrome c-reductase. The increased consumption of ATP is thought to be covered by a greater degradation of fat; the rise in free fatty acids in the serum and the increase of citrate synthase activity in the liver are an indication for this concept. By way of still unknown feedback mechanisms, a supression of the citrate cleavage enzyme occurs and, consequently, reduced lipogenesis results. The decrease in activity of the glycolytic key enzymes, fructose-6-phosphate kinase und pyruvate kinase, leads to a delay of glucose degradation in the liver; the reduced acetyl-CoA requirement which may be attributed to the inhibition of lipogenesis, is reflected in this.

The studies show that both antipodes of compound CH 13437 have a similar influence on the liver metabolism and consequently have an equally strong hypolipidaemic effect which is in direct correspondence with the one shown be the racemate.

Zusammenfassung. Die optischen Antipoden der hypolipidämisch wirkenden Substanz 2-Methyl-2-[*p*-(1, 2, 3, 4-tetrahydro-1-naphthyl)-phenoxy]-propionsäure (CH 13437) zeigen im Rattenversuch gleichstarke hypolipidämische Wirkung und gleichartige Aktivitätsänderungen von Leberenzymen des Kohlenhydrat- und Lipidstoffwechsels.

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Sympathetic Influence on Cerebral Blood Volume: Time-Dependent Effect of Decentralization of the Superior Cervical Ganglia in Mice

Although the pial arterial system receives a very extensive sympathetic innervation[1–5], the significance of the neural influence on brain circulation is highly controversial. One reason is that the results even of basic experiments, such as postganglionic denervation, appear conflicting because little attention is usually paid to the length of time that has elapsed after the operation. It has recently been shown[6] that the cerebral blood volume (CBV) of mice varies markedly depending on the time following bilateral excision of superior cervical sympathetic ganglia. Shortly after operation there is a leakage of the noradrenaline transmitter from the degenerating nerve terminals with an accompanying activation of the vascular receptor (the CBV was found to be reduced by 28%). When the transmitter has disappeared from the degenerating terminals, the neural influence on the vessels is abolished (the blood volume was increased by 34% compared to unoperated controls). About 2 weeks later, a pronounced denervation supersensitivity of the vascular receptors to circulating catecholamines develops (the CBV became normal or even subnormal).

Another circumstance giving the impression of inconsistent results after denervation is that a difference in the effects of pre- and postganglionic operation is usually not fully considered. The present report shows that the time-dependent changes in CBV are significantly different when

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