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The Molecular Structure of 2α-Hydroxyneoanisatin and Structure–Activity Relationships among Convulsant Sesquiterpenes of the *seco*-Prezizaane and Picrotoxane Types

Thomas J. Schmidt, [a,*] Emi Okuyama [b] and Frank R. Fronczek [c]

[a]*Institut für Pharmazeutische Biologie der Heinrich-Heine-Universität Düsseldorf, Universitätsstrasse 1, D-40225 Düsseldorf, Germany*
[b]*Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-Cho, Inage-Ku, Chiba 263-8522, Japan*
[c]*Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, USA*

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Abstract—The molecular structure of 2α-hydroxyneoanisatin, a positional isomer of the potent neurotoxin anisatin, was determined by X-ray crystallographic analysis. This compound and four further *seco*-prezizaane type sesquiterpene lactones previously isolated from *Illicium floridanum*, which represent different structural types with respect to the mode of cyclisation, did not induce anisatin/picrotoxinin-like convulsions in mice. Based on these results and literature data for other *seco*-prezizaanes, structural requirements for convulsant activity are discussed. Comparison of the three dimensional molecular shape and electrostatic properties of active and inactive *seco*-prezizaane type lactones with compounds of the picrotoxane type resulted in the identification of a common pharmacophore structure for these different skeletal classes of convulsant natural products.

Introduction

From fruits and leaves of *Illicium floridanum* (Star bush, American Star Anise; Illiciaceae), nine sesquiterpene lactones of the *seco*-prezizaane type were previously isolated.[1,2] Compounds of this type, whose occurrence is restricted to the genus *Illicium,* are of pharmacological and toxicological interest because some representatives are highly potent neurotoxins causing epileptiform convulsions followed by death through exhaustion, respiratory paralysis and hypoxia. The LD_{50} of the most prominent compound of this type, anisatin (**1**), a constituent of *Illicium anisatum* [3] and also of *Illicium floridanum*,[2] was determined at 1 mg/kg mouse, i.p. and p.o.[4,5] The activities of further highly active structural analogues such as neoanisatin (**3**) and the veranisatins (**4**–**6**) were shown to be similarly high.[4,5]

Anisatin has been demonstrated to possess a mechanism of action identical with that of picrotoxinin (**11**), a similarly toxic (LD_{50} at 3 mg/kg, mouse, i.p.)[6] convulsant sesquiterpene lactone of a different skeletal class isolated from seeds of *Anamirta cocculus* (Fish berry plant) and further related Menispermaceae.[7] The mechanism of action by which picrotoxinin exerts its convulsant activity has been studied in detail. It was shown to be a non-competitive allosteric inhibitor at $GABA_A$-receptor coupled chloride ionophores that mediate postsynaptic inhibition in the CNS of many different classes of organisms (see Refs 8–10 and literature cited there). It was found to bind to a specific site which is distinct from the $GABA_A$-receptor itself and from the benzodiazepine binding site, which is located inside the chloride channel pore. Here, binding of picrotoxinin leads to a decrease in GABA-induced Cl^- influx that normally causes a hyperpolarization at the postsynaptic neuron thus modulating the intensity of excitatory impulses. Repression of this inhibitory mechanism by picrotoxinin results in an overflow of excitatory neuronal impulses and, consequently, in lethal convulsions.

Anisatin, as mentioned above, has been demonstrated to act by the same mechanism of action as picrotoxinin[11–14] and experimental evidence indicates that it may bind to the same site at the channel protein.

In the present study, the X-ray crystal and molecular structure of 2α-hydroxyneoanisatin (**2**), a positional isomer of anisatin is reported. This compound and four further *seco*-prezizaanolides (**7**–**10**) with variable structural features isolated from *I. floridanum* [1,2] were tested in vivo for their capability to induce anisatin/picrotoxinin-like convulsions in mice in order to gain information on the structural requirements for convulsant activity.

Key words: Natural products; terpenes; X-ray; crystal structure; molecular modelling; convulsants; GABA antagonists.
* Corresponding author. Tel.: +49-211-811-4179; fax: +49-211-811-1923; e-mail: schmidt@uni-duesseldorf.de

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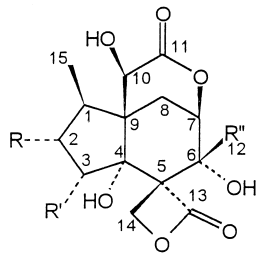
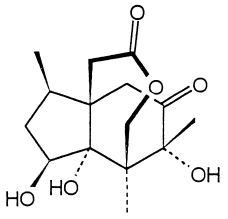


	R	R‘	R‘‘
1	H	OH	CH_3
2	OH	H	CH_3
3	H	H	CH_3
4	H	H	CH_2OCH_3
5	H	H	$COOCH_3$
6	H	OH	$COOCH_3$



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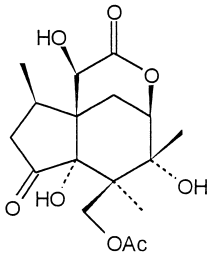

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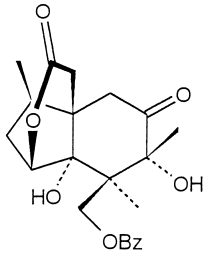

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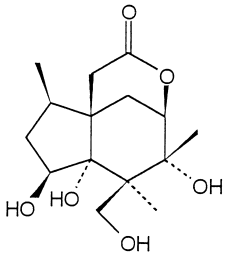

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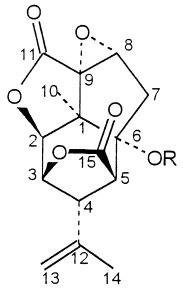
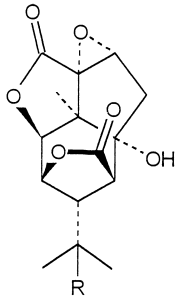
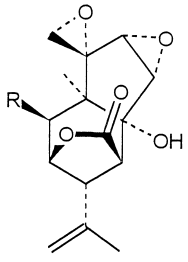


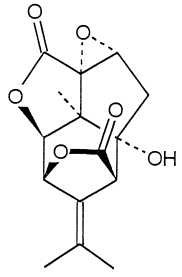
	R
11	H
12	Ac



	R
13	H
14	OH



	R
15	OH
16	H



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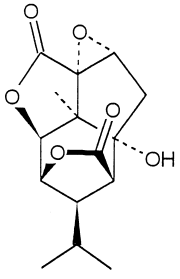

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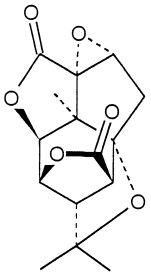

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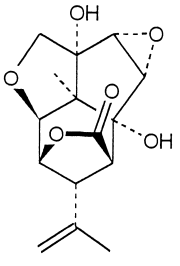

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(Ac = acetyl, Bz = benzoyl)

Based on computer molecular models, similarities in the three-dimensional shape and charge distribution of active convulsant derivatives of the anisatin- and picrotoxinin type are shown which result in the proposal of a common pharmacophore structure for these two structurally different classes of sesquiterpenes.

Results and Discussion

The structure of the isomer of anisatin (**1**), (−)-2α-hydroxyneoanisatin (**2**), was first described by Kouno et al., who isolated this compound from fruits of *I. anisatum*.[15] In a recent phytochemical investigation of *I. floridanum* Ellis, **2** was isolated from fruits and leaves of this North American species where it occurs together with large amounts of anisatin and a variety of further *seco*-prezizaane sesquiterpenes.[2] The molecular structure of **2** was determined by X-ray crystallographic analysis (Fig. 1, Tables 1 and 2) and found to be very similar to that of **1** as reported by Wong et al. in 1988,[16] who, however, depicted the molecule in the (+)-enantiomeric form, whereas the correct absolute stereochemistry of the naturally occurring (−)-enantiomer was determined two years later by total synthesis.[17] Two conformers of the **2**-molecule are observed in the unit cell (A and B, Fig. 1), which differ slightly in the orientation of the proton of the C-2α-OH group. Two independent molecules of **2** are observed in the asymmetric unit (A and B, Fig. 1). The two conformers are nearly identical, including the OH groups, which results from the fact that the hydrogen-bonding environments of the A and B molecules are also nearly identical. An intricate network of O-H...O hydrogen bonds exists, involving A...A, B...B, and A...B intermolecular interactions, as well as an O6-H...O5 intramolecular hydrogen bond in both molecules. The O5...O6 distance is 2.6167(12) Å in the A molecule and 2.6207(13) Å in the B molecule.

Anisatin **1** and its 3-deoxy derivative neoanisatin **3** as well as the veranisatins A-C (**4**–**6**) are potent neurotoxins causing lethal convusions at an LD_{50} of 1–3 mg/kg mouse p.o. The presence or absence of the α-oriented hydroxy function at C-3 thus obviously does not significantly influence the convulsant activity. It was therefore of interest to test the convulsant activity of **2** whose structure differs from the active compounds only in the OH-group at C-2. After p.o. application to mice, **2** did not cause convulsions or lethal toxicity at a dose of 3 mg/kg. A decrease of body temperature normally accompanies anisatin-like convulsions,[5] and a slight hypothermic effect could be observed at this dose indicating that the compound might be active as a convulsant at higher concentrations which, however, could not be tested due to the limited amount of the compound. It is a noteworthy finding that the shift of the hydroxy function from C-3 in **1** to C-2 in **2** causes such a drastic decrease in activity, while replacement of the OH group by a hydrogen in **3** does not show such an effect. In **1**, the C-3α-OH group is able to engage in an intramolecular hydrogen bond with the oxygen at C-4, while this is not possible for the C-2α-OH group of **2**. This shift in the position of the OH group renders **2** much more polar than **1**. The TLC-Rf value on silica

Table 1. Crystal data and X-ray data collection parameters for compound **2**

Formula	$C_{15}H_{20}O_8$
FW	328.32
Crystal system	Monoclinic
Space group	$P2_1$
Cell constants	
a,Å	7.8371(5)
b,Å	14.8315(9)
c,Å	13.0926(7)
β, deg	101.937(5)
V,Å^3	1488.9(3)
Z	4
Dc, g cm^{-3}	1.465
μ mm^{-1}	0.972
Temp. °C	26
Crystal size, mm	0.47×0.35×0.13
Radiation	CuKα(λ = 1.54184 Å)
θ limits, deg	2.5–75.0
Min. transmission, %	88.9
Octants collected	h ±k ±1
Unique data	6104
Observed data	6047
Criterion	I > 1σ(I)
Variables	543
R	0.031
R_w	0.040
Resid. density, e$^-$Å^{-3}	0.46
Extinction	3.07(8)×10^{-6}
Hydrogen atoms	Refined, isotropic

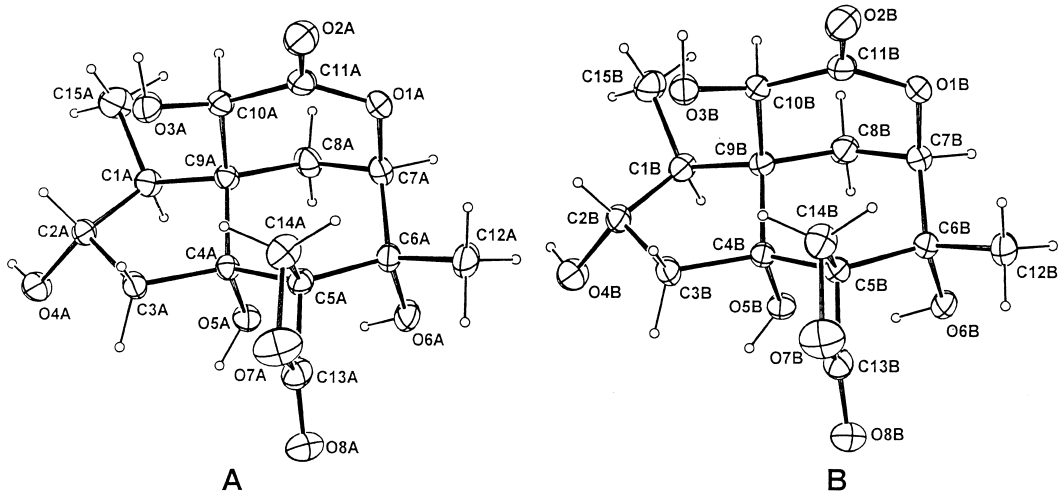


Figure 1. Molecular structures of the two conformers A and B of compound **2** as present in the asymmetric unit cell.

Table 2. Fractional coordinates for **2**

Atom	*x*	*y*	*z*	U_{eq}(Å^2)[a]
O1(A)	0.6592(1)	0	0.45231(8)	0.0484(5)
O2(A)	0.4776(2)	−0.10082(8)	0.37021(8)	0.0556(5)
O3(A)	0.2458(1)	−0.08007(7)	0.49756(8)	0.0426(5)
O4(A)	0.0791(1)	0.08201(8)	0.73276(8)	0.0484(5)
O5(A)	0.4023(1)	0.18754(7)	0.63159(7)	0.0347(4)
O6(A)	0.6445(1)	0.23578(7)	0.53181(8)	0.0446(5)
O7(A)	0.1915(2)	0.1768(1)	0.32091(9)	0.0572(7)
O8(A)	0.2585(2)	0.30234(8)	0.4217(1)	0.0559(6)
C1(A)	0.3536(2)	0.0159(1)	0.70000(9)	0.0345(6)
C2(A)	0.1592(2)	0.03669(9)	0.65785(9)	0.0337(5)
C3(A)	0.1498(2)	0.0978(1)	0.5614(1)	0.0317(5)
C4(A)	0.3405(1)	0.11701(8)	0.55826(9)	0.0265(5)
C5(A)	0.3884(2)	0.14508(9)	0.45400(9)	0.0300(5)
C6(A)	0.5902(2)	0.15842(9)	0.4690(1)	0.0365(6)
C7(A)	0.6856(2)	0.0757(1)	0.5257(1)	0.0390(6)
C8(A)	0.6343(2)	0.0509(1)	0.6273(1)	0.0378(6)
C9(A)	0.4394(1)	0.03081(9)	0.60463(9)	0.0284(5)
C10(A)	0.4200(2)	−0.05356(9)	0.5348(1)	0.0345(6)
C11(A)	0.5168(2)	−0.0519(1)	0.4449(1)	0.0406(6)
C12(A)	0.6492(2)	0.1758(1)	0.3668(1)	0.0534(7)
C13(A)	0.2781(2)	0.2247(1)	0.4038(1)	0.0417(6)
C14(A)	0.2928(2)	0.0947(1)	0.3558(1)	0.0409(7)
C15(A)	0.3862(2)	−0.0758(1)	0.7535(1)	0.0545(8)
O1(B)	0.9931(2)	−0.09812(7)	−0.11655(8)	0.0446(5)
O2(B)	1.1722(2)	0.00737(8)	−0.04466(9)	0.0608(6)
O3(B)	1.0821(1)	−0.00716(7)	0.14589(7)	0.0421(5)
O4(B)	0.8296(1)	−0.17561(8)	0.34470(8)	0.0541(5)
O5(B)	0.8664(1)	−0.28329(7)	0.11969(7)	0.0357(4)
O6(B)	0.9217(1)	−0.33462(7)	−0.06224(7)	0.0381(4)
O7(B)	1.3868(1)	−0.2634(1)	0.1209(1)	0.0562(7)
O8(B)	1.2261(2)	−0.39116(8)	0.12284(9)	0.0507(6)
C1(B)	0.7677(2)	−0.11206(9)	0.1656(1)	0.0368(6)
C2(B)	0.9017(2)	−0.1279(1)	0.2679(1)	0.0362(6)
C3(B)	1.0514(2)	−0.1840(1)	0.24036(9)	0.0338(5)
C4(B)	0.9861(2)	−0.20907(8)	0.12519(9)	0.0280(5)
C5(B)	1.1184(2)	−0.23569(9)	0.05827(9)	0.0306(5)
C6(B)	1.0226(2)	−0.25401(9)	−0.05741(9)	0.0324(6)
C7(B)	0.8928(2)	−0.1776(1)	−0.0986(1)	0.0362(6)
C8(B)	0.7672(2)	−0.1546(1)	−0.0288(1)	0.0384(6)
C9(B)	0.8721(2)	−0.12773(9)	0.0788(1)	0.0300(5)
C10(B)	0.9670(2)	−0.04102(9)	0.0574(1)	0.0336(5)
C11(B)	1.0564(2)	−0.0435(1)	−0.0361(1)	0.0419(6)
C12(B)	1.1493(2)	−0.2700(1)	−0.1295(1)	0.0452(7)
C13(B)	1.2381(2)	−0.3134(1)	0.1041(1)	0.0406(6)
C14(B)	1.2909(2)	−0.1818(1)	0.0766(1)	0.0427(6)
C15(B)	0.6685(2)	−0.0232(1)	0.1613(1)	0.0517(7)

[a] $U_{eq} = (1/3)\sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a_i}.\mathbf{a_j}$.

plates in cyclohexane/EtOAc 6:4 of **2** in relation to that of **1** is only 0.12. Increased polarity and hydrophilicity might thus be the reason for the dramatic decrease in activity, considering that these compounds are quite polar in general and have to pass the lipophilic blood-brain barrier to exert their convulsant effect.

To gain further insight into structural requirements for convulsant activity, four further *seco*-prezizaanolides previously isolated from *I. floridanum*, namely, pseudoanisatin (**7**),[1,2] 14-acetoxy-3-oxofloridanolide (**8**),[2] 7-deoxy-7-oxodunnianin (**9**)[1] and 3β,14-dihydroxy-10-deoxyfloridanolide (**10**)[2] were also tested for convulsant activity. None of these compounds, representing different modes of cyclisation and substitution, was found to be active at a dose of 50 mg/kg (p.o). Neither convulsions nor a change in body temperature could be observed.

Thus, all *seco*-prezizaanes found highly active as convulsants so far (**1, 3–6**) possess the same mode of cyclisation as anisatin, including both the β-lactone and the α-hydroxylated δ-lactone ring. Changes in the structure of the lactone rings, e.g. formation of an 11,14-ε-lactone or an 11,3-δ-lactone as in **7** and **9** or mutation of the 13,14-β-lactone to 13-methyl,14-hydroxy or –O-acyl substituents in **8** and **10** lead to a complete loss of activity. The β-lactone ring thus appears to represent an essential structural prerequisite for high convulsant activity.[18] Since all β-lactone derivatives also contain the 10-hydroxy-7,11-δ-lactone structure, it cannot be judged whether the hydroxy group at C-10 is also indispensable, solely by inspection of the structure and activity data of *seco*-prezizaanes. Evidence arising from the comparison with picrotoxanes indicating that this part of the structure is indeed essential for activity, is presented below. Substitution at C-12 appears to be without effect on activity, since veranisatins A-C (**4–6**) are active in the same concentration range as anisatin itself.[4,5] The high activity of the veranisatins, moreover, indicates that sterically more demanding substituents at C-12 do not influence activity so that it may be expected that the receptor structure does not occupy space in this area.

The influence of the C-2 hydroxy function of **2** which dramatically decreases the activity in comparison with **1** and **3**, as mentioned above, may be attributable to its effect on the compound's lipophilicity, or, alternatively to an unfavourable interaction of this more exposed OH-group with the receptor.

Structure–activity relationship studies on picrotoxinin derivatives have revealed information on the structural requirements for convulsant activity within this group.[6,19,20]

The γ-lactone ring fused between C-3 and C-5 in *cis*-orientation to the fused carbocyclic system [6] and a free hydroxy group at C-6 [6,20] are essential for activity. Furthermore, the alkyl sidechain must be oriented *trans* to the lactone ring; a *cis* oriented side chain as in β-dihydropicrotoxinin (**18**) renders the compound inactive.[6,19] Hydrogenation of the side chain double bond leads only to a fivefold decrease in activity (α-dihydropicrotoxinin, **13**) while hydration, i.e. introduction of a tertiary hydroxy group, leads to a 30–50-fold reduction (picrotin, **14**) and shift of the double bond to the $\Delta^{4(12)}$ position (neo-picrotoxinin, **17**) results in complete loss of activity.[6] Moreover, masking of the C-6 OH function by acetylation (picrotoxinin acetate, **12**) or by formation of an ether bridge with the side chain carbon C-12 (anhydropicrotin, **19**) leads to loss of activity [6,20] which indicates that this OH acts as a H-bond donor. The picrotoxanes tutin (**15**) and coriamyrtin (**16**) lacking the lactone ring fused between C-2 and C-11 and possessing two epoxide groups instead of one, are highly active [6] so that the second lactone function of **11** appears to be unimportant for activity. From the observation that corianin (**20**), a derivative of coriamyrtin that lacks the extracyclic 9,11-epoxide moiety is inactive,[21] it may be concluded firstly, that the presence of an epoxide group in this region is indispensable

for activity, and, secondly, that it must be the extracyclic 9,11-epoxy group in coriamyrtin and tutin that plays the same role as the 8,9-epoxy group in picrotoxinin. Taking into account the structural features shared by all highly active picrotoxanes and the structural difference of less or inactive derivatives,[22] a pharmacophore structure may be deduced that comprises the isopropenyl substituted γ-lactone ring, the C-6-OH group and an α-oriented epoxide function attached either to C-8 and C-9 or to C-9 and C-11.

Since experimental evidence indicates that both groups of convulsants bind to a common receptor site,[12,13] the question arose whether their structures contain a common pharmacophore pattern. To this end, the 3D-structure of **1** was superimposed with the pharmacophore of picrotoxinin in a variety of different arrangements. Quite noteworthy, an overlay of the δ-lactone group of **1** with the 3,5-lactone of **11** led to an arrangement in which also the oxygen in the β-lactone ring of **1** and the epoxide oxygen of **11** are in an almost identical position (Fig. 2A). The triangle formed by these oxygen atoms in both molecules is nearly identical with respect to all interatomic distances and angles (RMS distance for overlay of the three mentioned oxygen atoms 0.039 Å), so that possibilities for hydrogen bonding interactions with the receptor are very similar for these three acceptors. Moreover, in this alignment, the methyl group at C-1 of **1** is in a position very close to the methyl of picrotoxinin's isopropenyl group. The 'outward' sides of the lactone rings and the lipophilic substituents (isopropenyl in **11** and C-9, C-1 and C-15 of the methylcyclopentane in **1**, large ellipsoid marked on the left in Fig. 1) form a relatively flat surface area that would allow for very similar hydrophobic interactions with a non-polar protein region. The distance between the oxygens of the C-6 OH group of **11** and the C-10 OH of **1**, moreover, although being 1.6 Å in this alignment, is small enough to allow for interaction of the respective OH protons with the same H-bond acceptor on the receptor. Thus, a four-point RMS overlay (comprising the two lactone- and the epoxide oxygen as well as the mentioned methyl groups) of the structures of picrotoxinin and anisatin was considered to represent their most likely orientation at a common binding site. Figure 2B shows the electrostatic potential mapped on a total charge density surface. The electrostatic properties of both convulsants are very similar in the mentioned regions (areas marked by yellow ellipsoids). The common receptor structure could thus consist of a flat hydrophobic structure element, possibly an aromatic amino acid side chain, interacting with the large hydrophobic region on the outward side of the lactone rings, and hydrogen bond donors interacting with the lactone- and epoxide/β-lactone oxygens. A fourth group, capable of accepting a hydrogen bond from C-6/C-10 OH groups should, furthermore, be involved.

The mentioned structural features should, therefore, be shared by all active convulsants within these groups, while weakly active or inactive compounds should show discrepancies from the common pharmacophore in one

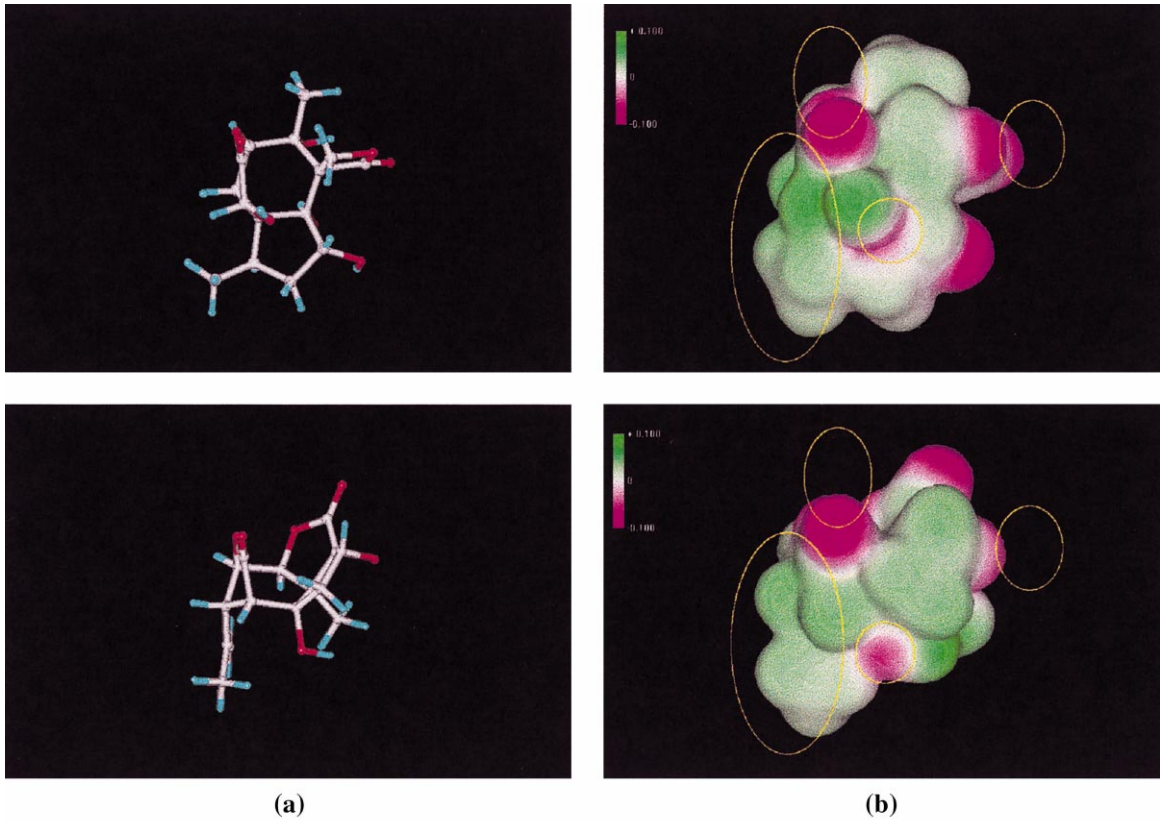
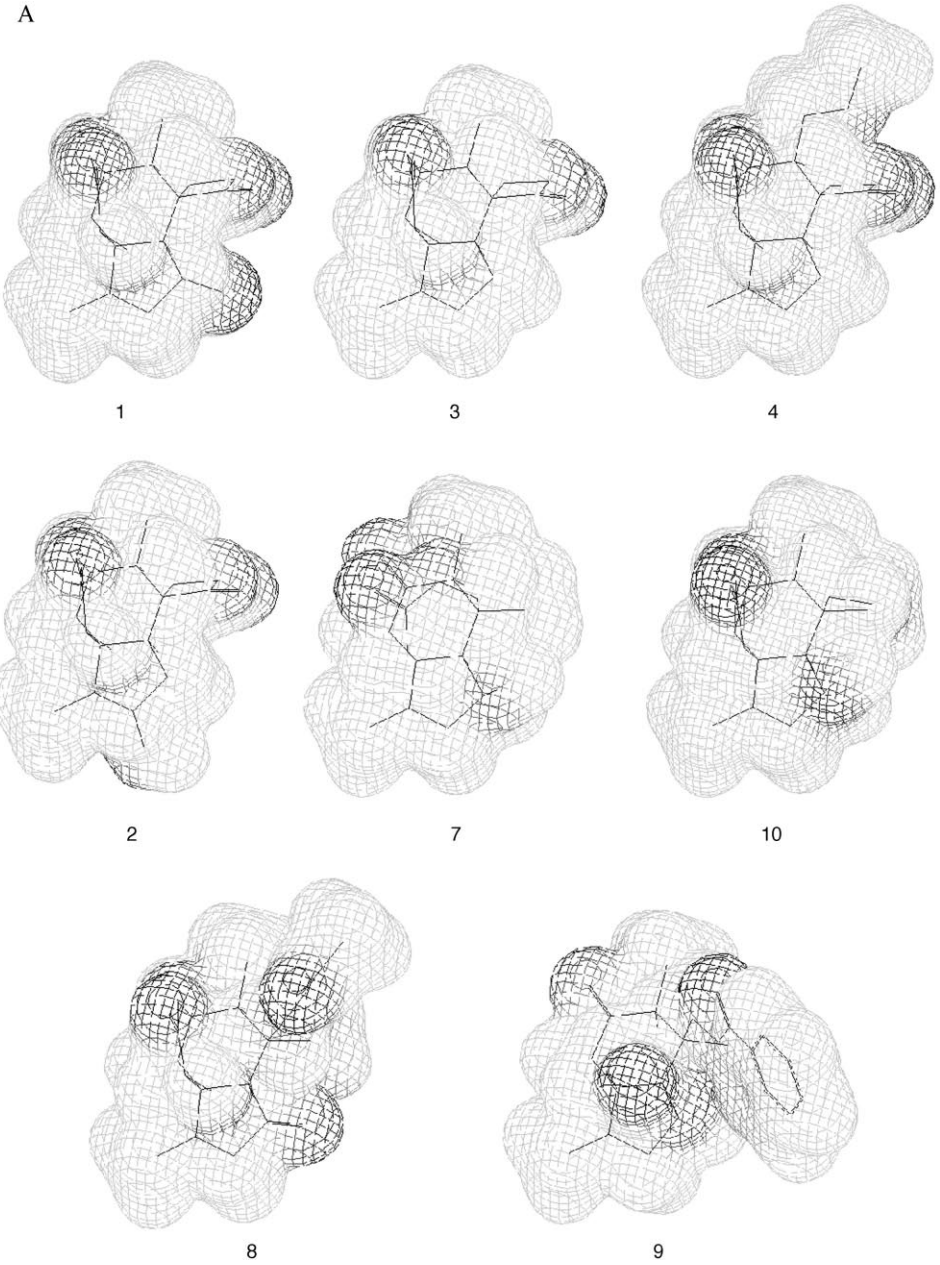


Figure 2. Molecular models of anisatin (**1**, top) and picrotoxinin (**11**, bottom) showing the arrangement of the molecular skeleta (**A**) and the resulting molecular electrostatic potential between −0.1 and 0.1 mapped on a surface at which the total charge density is 0.0025 (**B**) (values expressed in atomic units e/a_0 and e/a_0^3, respectively). Yellow ellipsoids mark areas of high similarity that are likely to determine binding to a common receptor site.

or more respects. To test this hypothesis, the AM1 minimized 3D-structures of a variety of known active and inactive picrotoxane and *seco*-prezizaane derivatives were aligned and their electrostatic potential maps created in the same way as described above for **1** and **11** (Fig. 3; alignment and viewing angle identical with that of Fig. 2). Of the active picrotoxinin analogues (**13**–**16**), **15** and **16** possess essentially the same characteristics as **11** with respect to the hydrophobic site (left side in Fig. 2) and show only a slight difference in the distribution of negative charge on the right side, where they possess two epoxide groups, while **11** possesses only one. In **13**, which is about 4.5 times less active than **11**, the isopropyl group causes a slight increase of steric bulk at the lower end of the hydrophobic interaction site which, however, may be compensated by rotation around the 4,12-bond. A similar shape is exhibited by **14**, which, however, is about 50 times less active than **11**. This loss of activity may be explained by the observation that an intramolecular H-bond between the OH- groups at C-12 and C-6 can be formed, hindering both the rotation of the isopropyl group (which would be necessary to eliminate steric bulk in the same way as in **13**) and of C-6-OH which must form a H-bond to the receptor site. Among the inactive picrotoxanes, neopicrotoxinin (**17**) shows increased steric bulk at the side chain part of the hydrophobic site and, in this case, rotation around the C-4-C-12 bond is not possible. Even more dramatic steric hindrance is observed in β-dihydropicrotoxinin (**18**), where the isopropyl side chain is oriented *cis* relative to the 3,5-lactone ring. The observation that **19** is inactive is explained by the fact that its C-6 oxygen



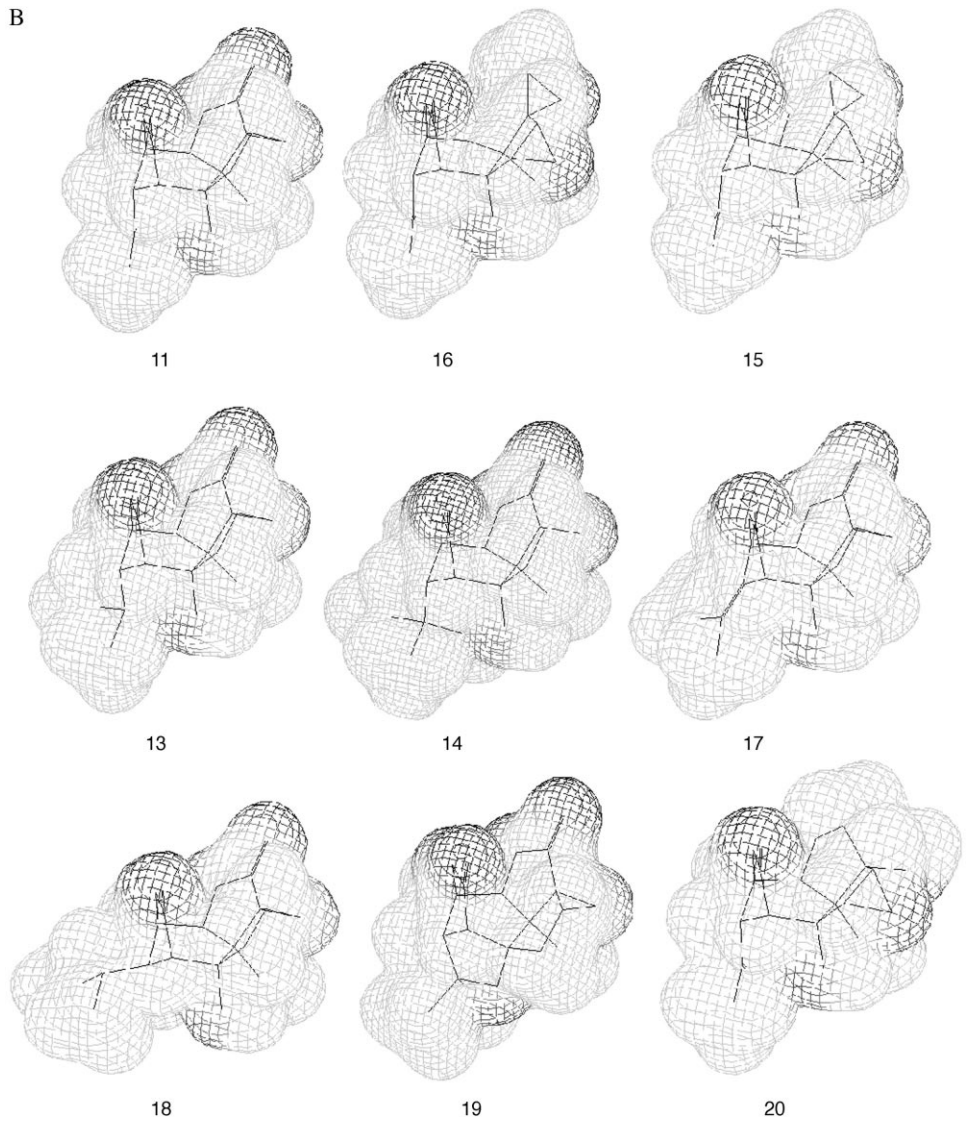


Figure 3. Electrostatic potential maps of convulsant and inactive *seco*-prezizaane (**A**) and pictrotoxane (**B**) sesquiterpene lactones. Viewing angle and calculation details identical with those of Figure 2; negative potential is indicated by dark lines. For implications on convulsant activity see text.

function is engaged in a cyclic ether bridge and is thus not capable of donating a hydrogen bond, since the other molecular features (steric bulk and charge distribution) are very similar to those of **11**.

In the group of anisatin analogues, the active derivatives **3** and **4** show the same characteristics as **1** with respect to the pharmacophoric arrangement, the absence of the C-3-OH group in both and the additional substituent at C-12 in **4** being without influence. The shape and charge distribution of compound **2** is very similar to the highly active convulsants and it remains to be shown in further studies whether this is due to an unfavourable interaction of the 2-OH group with the receptor or merely by its higher polarity which impairs transport to the target.

In the inactive derivatives, **7**–**10**, the hydrophobic part on the left side possesses largely the same shape and size as in the active compounds. The C-14-O-acyl moieties in **8** and **9** lead to drastic changes in molecular shape on the right side. In **7** and **10**, the crucial difference from the active convulsants is observed in the lack of negative charge on the right side which renders them unable to accept a hydrogen bond analogous to that engaging the β-lactone ring oxygen of the active compounds. Moreover, **7** and **10** also lack the C-10-OH group. Their incapability to donate a H-bond to the receptor site, as mentioned above for **19**, further explains their lack of convulsant activity.

Conclusions

From these considerations it becomes clear that although the molecules of the two different groups of convulsants do not show much constitutional similarity, their three-dimensional shape and charge distribution is very similar in the regions identified as essential for activity. It is, therefore, quite plausible to assume an identical site and mode of binding at the GABA-gated chloride channel protein. Further examinations with the aim of generating a pseudoreceptor structure based on

the pharmacophore model presented in this study are in progress. In combination with receptor binding studies, such a pseudoreceptor may become useful to carry out 3D-QSAR investigations for convulsants and will be of interest for future research on the function of the picrotoxinin binding site on GABA-gated chloride channels.

Experimental

X-ray crystallography

X-ray diffraction data [23] for **2** were collected on an Enraf-Nonius CAD4 diffractometer equipped with Cu$K\alpha$ radiation ($\lambda = 1.54184$ Å) and a graphite monochromator. Friedel-related data were collected. Data reduction included corrections for background, Lorentz, polarization, and absorption effects. Absorption corrections were based on ψ scans. The structure was solved by direct methods and refined using the MolEN programs.[24] Refinement was by full-matrix least squares, with neutral-atom scattering factors and anomalous dispersion terms. Weights were $w = 4Fo^2[^2(Fo^2) + 0.0004\ Fo^4]^{-1}$. All non-hydrogen atoms were refined anisotropically while hydrogen atoms were refined isotropically. Crystal data, final R values, and other details are included in Table 1, final atomic coordinates in Table 2. Refinement with the reported (expected) absolute configuration yielded $R = 0.0310$, $Rw = 0.0402$, while the opposite configuration yielded $R = 0.0313$, $Rw = 0.0406$.

Animal testing

Male ddy mice (5 w, 27–32 g) were purchased from Japan SLC (Hamamatsu, Japan) and were used in the experiments after having been kept in an animal room for about 1 week under standard conditions (22 ± 1°C, circadian cycle of 12 h light and 12 h darkness), standard diet and water *ad libitum*. In each experiment, samples were suspended in 5% arabic gum. The vehicle without sample was used as control and aminopyrine (100 mg/kg) served as positive control. The drugs were administered orally. Rectal temperature was measured up to 4 h after oral administration of samples with a thermister (Takara Instruments, Japan) and the animals' behaviour was constantly observed for the occurrence of convulsions.

Molecular modelling

All molecular models were generated with Hyperchem 5.1. Models of compounds (**1**,[16] **2**, **7**,[25] **8**,[2] **9**[1], **10**[2] and **11**[26]) were created by using X-ray coordinates (input in PCModel v. 4) as starting structures (structure of **1**[16] was transformed to the enantiomer), which were energy minimised using the AM1 hamiltonian and the Polak–Ribiere (conjugate gradient) minimisation algorithm as implemented in Hyperchem. Termination criterion was an RMS gradient < 0.01 kcal mol^{-1} A^{-1}. The other structures were modelled by applying the appropriate changes to the X-ray based models, force field (MM+) preminimised, and subsequently energy minimised as described above.

The structures of anisatin and picrotoxinin were superimposed by RMS fitting the oxygen atoms of the δ-lactone, the β-lactone ring oxygen and the C-15 methyl group of **1** with the oxygen atoms of the γ-lactone connecting C-3 and C-5, the epoxide oxygen and the C-14 methyl group of **11**.

Structural alignment of the other compounds was carried out by RMS overlay of the perhydroindane system with that of anisatin (*seco*-prezizaanes) and with that of picrotoxinin (picrotoxanes), respectively.

The electrostatic surface maps in Figures 2 and 3 represent the electrostatic potential between −0.1 and 0.1 au (e/a_0) on a shell of 0.0025 au (e/a_0^3) total charge density. They were calculated with a spacing increment between points of 0.3 Å.

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18. As a sole exception, neomajucin, containing a γ-lactone ring closed between C-13 and C-12 instead of the β-lactone, was shown to possess convulsant activity (LD_{50} at 12 mg/kg, mouse i.p.) which, however, is much weaker than that of the β-lactones. Neomajucin appears to represent an abnormality, since other majucin derivatives are inactive. See Kouno, I.;

Baba, N.; Hashimoto, M.; Kawano, N.; Takahashi, M.; Kaneto, H.; Yang, C.-S.; Sato, S. *Chem. Pharm. Bull.* **1989**, *37*, 2448 and Kouno, I.; Baba, N.; Hashimoto, M.; Kawano, N.; Takahashi, M.; Kaneto, H.; Yang, C.-S. *Chem. Pharm. Bull.* **1990**, *38*, 422.
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22. A number of nitrogen containing picrotoxane derivatives isolated from Dendrobium species, e.g. dendrobine, are also convulsants which, however, apparently possess a somewhat different mode of action since dendrobine has been shown to influence glycine- rather than GABA receptors (Curtis, D.R.; Duggan, A.W.; Felix, D.; Johnston, G.A.R. *Brain Res.* **1971**, *32*, 69 and Kudo, Y.; Tanaka, A.; Yamada, K. *Br. J. Pharmac.* **1983**, 78, 709); These derivatives were, therefore, not taken into account in the present study. A recent QSAR study on picrodendrins, a group of GABA antagonistic norditerpenes isolated from the Euphorbiacea *Picrodendron baccatum*, whose carbon skeleton is for the major part identical with that of the picrotoxanes, has shown the importance of basically the same structural features with respect to those molecule parts which these compounds share with the picrotoxanes (Ozoe, Y.; Akamatsu, M.; Higata, T.; Ikeda, I.; Mochida, K.; Koike, K.; Ohmoto, T.; Nikaido, T. *Bioorg. Med. Chem.* **1998**, *6*, 481). The most active representative of this series, picrodendrin Q, possesses an isopropyl group at C-4 in *trans* orientation to the 3,5-lactone ring (analogous to α-dihydropicrotoxinin) and, moreover, shares with the active picrotoxanes the C-6-OH group and an α-oriented oxygen function at C-13 (equivalent to C-9 in the picrotoxanes). Apart from the influence of the additional carbon and oxygen atoms which apparently lead to a somewhat altered mode of binding of the picrodendrins (picrodendrin Q is more active than picrotoxinin in spite of possessing an isopropyl instead of an isopropenyl group), introduction of OH groups at C-4 or C-8 (equivalent to C-4 and C-12 of the picrotoxanes, respectively) leads to a decrease in activity.
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