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PARTIAL STRUCTURES OF THE FUNGAL TOXIN AFLATREM, METHYL-SUBSTITUTED 6,8-DIOXABICYCLO[3.2.1]OCTAN-2-ONES HAVE ANTICONVULSANT ACTIVITY

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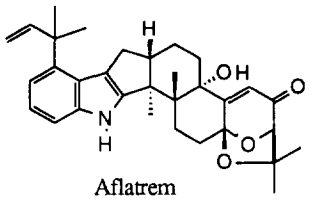
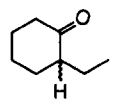
Abstract: 4,7,7-Trimethyl-6,8-dioxabicyclo[3.2.1]octan-2-one was found to be an effective anticonvulsant (ED_{50} = 131 mg/kg) against pentylenetetrazole-induced seizures in mice. Enantioselectively was observed in the actions of the (+)- and (-)-enantiomers as anticonvulsants and as displacers of [^{35}S]-TPBS, a ligand for the picrotoxin site on $GABA_A$ receptors. The (-)- enantiomer was slightly more potent in both biological assays.

Aflatrem is a fungal toxin belonging to the paspalitrem family of toxins. This compound affects animal behavior in a complex manner. Mice given this toxin are initially inactive. When the animals are stimulated to move, their movements are accompanied by tremors of the entire body. At higher doses of aflatrem, the tremors are followed by convulsions, and sometimes, death.[1] The pharmacological basis for the behavioral effects of aflatrem is not understood completely. There is a report that the compound is a neurotoxin,[2] and additional reports of both negative[3] and positive[4] allosteric effects of aflatrem on GABA (γ-aminobutyric acid) action at $GABA_A$ receptors.

Based on electrophysiological results obtained from *Xenopus* oocytes transfected with mRNA for chicken brain $GABA_A$ receptors, it was suggested that the initial inactivity of mice given this toxin resulted from aflatrem-mediated potentiation of inhibitory GABAergic neurotransmission. The tremors later observed in the mice were attributed to an overriding increase in excitatory glutamatergic neurotransmission resulting from an aflatrem-mediated enhancement in the release of glutamate and aspartate. It was further suggested that it might be possible to separate the different effects of aflatrem on the two different neurotransmitter systems by altering, in a manner not specified, the structure of aflatrem.[4]



Aflatrem



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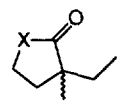


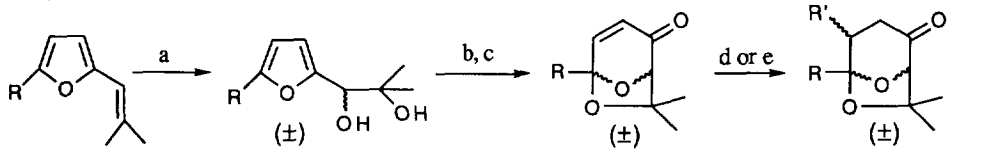
2 X = O
3 X = S

We have been engaged in extensive structure-activity studies of the neurological actions of alkyl-substituted γ-butyrolactones and related cyclic congeners. Compounds **1-3** shown above are representative of

many α-alkyl substituted compounds we have identified as having anticonvulsant activity against pentylenetetrazole-induced seizures in mice.[5-8] Electrophysiological and pharmacological studies of anticonvulsants **1-3** have shown that, like aflatrem, these compounds are positive allosteric modulators of GABA action at $GABA_A$ receptors.[9,10] The similarity in the actions of these anticonvulsants and aflatrem[4] on $GABA_A$ receptor function, and the similarity in the structures of these small cyclic molecules to the bicyclic enone portion of aflatrem suggested to us that partial structures of aflatrem might have anticonvulsant activity. We report here the synthesis of a group of methyl-substituted 6,8-dioxabicyclo[3.2.1]octan-2-ones, their anticonvulsant activity, and their ability to displace [^{35}S]-TBPS from the picrotoxin binding site on $GABA_A$ receptors. The (+)- and (-)-enantiomers of the most potent anticonvulsant compound (**13**, *vide infra*) were prepared and examined to investigate the effect of chirality on biological action.

Following a synthetic strategy described by Ali et al.[11] to prepare racemic 5,7,7-trimethyl-6,8-dioxabicyclo[3.2.1]oct-3-en-2-one (**9**), the enantiomeric mixtures of dioxabicyclic compounds **10-13** were obtained directly from enones **8** or **9** by either conjugate addition of Me_2CuLi or by catalytic hydrogenation of the double bond using palladium on activated charcoal (Scheme 1).[12] Starting with furfural or methylfurfural, compounds **4** and **5** were obtained *via* a Wittig reaction using isopropyltriphenylphosphonium iodide in 74% and 78% yield, respectively. A 1,2-dihydroxylation carried out with catalytic amounts of osmium tetroxide and N-methylmorpholine N-oxide as co-oxidant gave the diols **6** (76%) and **7** (70%). The enones **8** and **9** were synthesized using *m*-chloroperbenzoic acid to generate intermediate 6-hydroxy-β-pyrones[13] (structures not shown), which after removal of the reaction solvent and treatment of the crude product with *p*-TsOH and anhydrous $CuSO_4$ in benzene, gave compounds **8** (56%) and **9** (43%). The hydrogenation reactions (**8**→**10**; **9**→**11**) and the Michael addition reactions (**8**→**13**; **9**→**12**) gave quantitative yields of products. The Michael addition reaction proved to be stereoselective. The ^{1}H and ^{13}C NMR spectra of compounds **12** and **13** showed that each compound was a single diastereomer. An analysis of the proton-proton coupling constants for H-4 indicated that the 4-methyl group had the equatorial configuration in both compounds.[14]

Scheme 1



4 R = H
5 R = CH_3

6 R = H
7 R = CH_3

8 R = H
9 R= CH_3

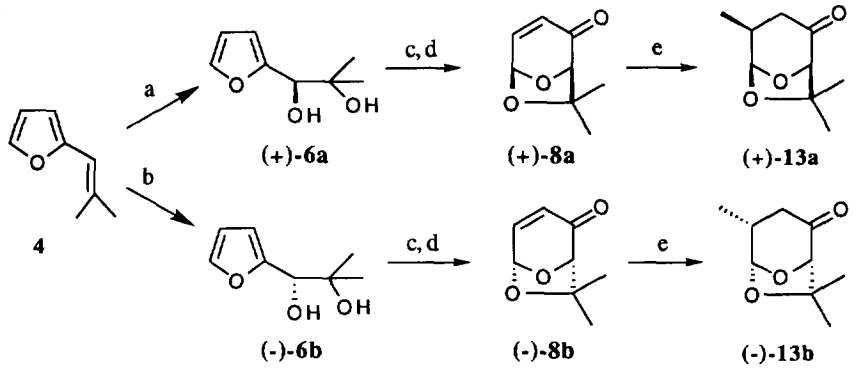
10 R = H, R' = H
11 R = CH_3, R' = H
12 R = CH_3, R' = CH_3
13 R = H, R' = CH_3

Reagents: a) OsO_4, NMO, acetone/water/*t*-BuOH; b) MCPBA, CH_2Cl_2;
c) TsOH, $CuSO_4$, Bz; d) R' = CH_3; MeLi, CuI, THF; e) R' = H; H_2, Pd/C.

The (+)- and (-)-enantiomers of compound **13**, the most potent bicyclic anticonvulsant (Table 1), were then synthesized to investigate the effect of chirality on biological activity. The enantiomerically pure compounds (+)-**13a** and (-)-**13b** were obtained from the corresponding enantiomerically pure vicinal diol precursors (+)-**6a** and (-)-**6b** (Scheme 2). Using the commercially available mixture of either dihydroquinine or dihydroquinidine with $K_3Fe(CN)_6$, K_2CO_3, and $K_2OsO_2(OH)_4$; (AD-mix α or AD-mix β),[15] reagents developed by Sharpless et al.[16] for catalytic asymmetric 1,2-dihydroxylation, it was possible to obtain the vicinal diols (+)-**6a** and (-)-**6b** in

>95% enantiomeric excess as evidenced by NMR experiments carried out in the presence of the chiral alcohol (*R*)-2,2,2-trifluoro-1-(9-anthryl)ethanol.[17,18] The absolute configuration of these diols was based initially on a mnemonic which entails top or bottom attack based on the substituents on the olefin and the type of ligand used.[16,19] The dihydroxypropylfurans (+)-**6a** and (-)-**6b** led to the formation of enones (+)-**8a** and (-)-**8b**, respectively, by following the same oxidation and dehydration procedures mentioned above. The cuprate addition using Me_2CuLi on (+)-**8a** and (-)-**8b** gave (+)-**13a** and (-)-**13b**, respectively.[21,22]

Scheme 2



Reagents:
a) $(DHQ)_2$-PHAL, $K_3Fe(CN)_6$, K_2CO_3, $K_2Os_2(OH)_4$, *t*-BuOH/H_2O; b) $(DHQD)_2$-PHAL, $K_3Fe(CN)_6$, K_2CO_3, $K_2Os_2(OH)_4$,*t*-BuOH/H_2O; c) MCPBA, CH_2Cl_2; d) TsOH, $CuSO_4$, Bz; e) MeLi, CuI, THF,

Compounds **8** and **10-13** did not exhibit convulsant properties in mice within a period of 30 min after injection. The enone precursor **8** was not an effective anticonvulsant against pentylenetetrazole-induced seizures even at doses in excess of the TD_{50} dose in the rotorod toxicity test (Table 1). We speculate that the potent toxicity of enone **8** is a consequence of it's reactivity as a Michael acceptor. Compounds **10-13** do not possess the enone moeity and all of these compounds had anticonvulsant activity at doses below those causing rotorod toxicity.

Compound **10**, displayed anticonvulsant activity with an ED_{50} = 192 mg/kg. When both the 4- and the 5-positions were substituted with a methyl group (compound **12**), the potency of the compound as an anticonvulsant increased (ED_{50} = 140 mg/kg). Substitution with a methyl group at only the 5-position (compound **11**) caused a decrease in anticonvulsant potency, whereas methyl group substitution on only the 4-position (compound **13**) increased anticonvulsant potency (ED_{50} = 131 mg/kg). Compounds (+)-**13a** and (-)-**13b** were then examined for their anticonvulsant activity. Both enantiomers displayed anticonvulsant activities, with (-)-**13b** displaying a better potency (ED_{50} = 110 mg/kg) than (+)-**13a** (ED_{50} = 144 mg/kg).

Since aflatrem and compounds **1-3** have been shown previously to displace [^{35}S]-TBPS, a radioligand for the picrotoxin binding site on the $GABA_A$ receptors,[3,7,9] the abilities of analogues **10-13**, (+)-**13a** and (-)-**13b** to displace [^{35}S]-TBPS were examined (Table 1). In comparison to aflatrem, all of the compounds were weak displacers of [^{35}S]-TBPS. However, compounds having a methyl group at the 4-position did have increased potency for [^{35}S]-TBPS displacement, just as they had increased anticonvulsant potency. When the effect of chirality was examined for compound **13**, it was found to exert a modest effect on [^{35}S]-TBPS displacement. Once again, however, the more potent displacer of [^{35}S]-TBPS was the more potent anticonvulsant. Thus, (-)-**13b** was more potent than (+)-**13a** in both bioassays. Methyl group substitution at

the 5-position (compound **11**) gave a compound that displaced [^{35}S]-TBPS as effectively as compound **13**, the 4-methyl substituted compound, but this compound was a less potent anticonvulsant than compound **13**.

Table 1. Anticonvulsant Activity, Neurotoxicity, and [^{35}S]-TBPS Binding Data

Compound No.	ED_{50}[a] (mg/kg)	TD_{50}[b] (mg/kg)	IC_{50}[c,d] (mM)
8	>300	<100	----
10	192	>300	5.09 ± 0.39
11	247	>300	3.13 ± 0.04
12	140	>300	2.31 ± 0.19
13	131	>300	3.02 ± 0.03
(+)-**13a**	145	----	3.09 ± 0.03
(-)-**13b**	110	----	2.85 ± 0.02*
Aflatrem[e]			0.0043 ± 0.1
1[f]	140	245	1.2 ± 0.1
2[g]	259	>500	2.3 ± 0.2
3[g]	128	244	0.33 ± 0.2

[a]Dose at which 50% of the mice were protected from clonic seizures induced by pentylenetetrazole (85 mg/kg). Except for compound **8**, a minimum of 4 doses of each compound dissolved in 30% polyethylene glycol was evaluated. A group of 6 animals was used at each dose tested. Other details of our evaluation method are described in ref 8.

[b]Dose at which 50% of the mice failed the rotorod toxicity test. See ref. 8 for other details of the test method.

[c]Binding data are presented as the mean ± SEM of three experiments performed in triplicate. Details of our binding assay are described in ref 8. The IC_{50} values determined for pentylenetetrazole and picrotoxinin were 759 ± 53 μM (n = 3) and 310 ± 40 nM, respectively.

[d]The ED_{50}, TD_{50}, and IC_{50} values were determined by log probit analysis of the dose-response data.[23]

[e]Result reported is from ref 3.

[f]Data reported are from ref 8.

[g]Data reported are from ref 7.

*p = .003 as compared to (+)-**13a**; t test.

The results reported show that partial structures of aflatrem have anticonvulsant activity and possibly new analogues containing other variations of the aflatrem structure will have even greater anticonvulsant potency. The low potency of the dioxabicyclic compounds described herein as displacers of [^{35}S]-TBPS indicates that these compounds, like the earlier prepared anticonvulsants **1-3** (see Table 1), have only weak interactions with the picrotoxin site on $GABA_A$ receptors. A comparison of the anticonvulsant potencies of compounds **3** and **13** to

their potencies as displacers of [^{35}S]-TBPS (Table 1) indicates that their anticonvulsant potencies do not correlate with their affinities for the picrotoxin site on $GABA_A$ receptors. This may indicate that the site(s) of action of compounds **3** and **13** on $GABA_A$ receptors are not identical. Additional binding and electrophysiological studies are in progress to investigate more fully the pharmacological basis for the anticonvulsant activities of the novel compounds reported here.

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12. All previously unknown compounds gave satisfactory elemental analyses for C, H and had IR, ^{1}H NMR [$CDCl_3$, TMS ($\delta = 0.00$)], and ^{13}C NMR spectra consistent with the structures given.
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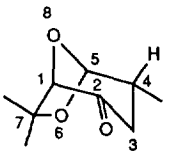
14. For compound **13**, the ^{1}H NMR spectrum shows H-3_{ax} as a dd (J_{gem} = 18.3 Hz, $J_{3a,4a}$ = 8.1 Hz) at δ 2.59, and H-3_{eq} appears as a dd (J_{gem} = 18.3 Hz, $J_{3e,4a}$ = 3.4 Hz) at δ 2.10. A NOESY experiment provided evidence for the chair conformation of the six-membered ring. An NOE was observed between H-3_{ax} and the endo-Me at position C-7.



15. The 1.4 g AD mix α or β necessary for the conversion of 1 mmol of the olefin contains 0.980 g of $K_3Fe(CN)_6$ (3.0 mmol), 0.410 g of K_2CO_3 (3.0 mmol), 0.0078 g of $(DHQ)_2$-PHAL (dihydroquinidine phthalazine for AD mix α) or $(DHQD)_2$-PHAL (dihydroquinine phthalazine for AD mix β) (0.01 mmol), and 0.00074 g of $K_2OsO_2(OH)_4$ (0.002 mmol).
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18. When the ^{1}H NMR spectra were recorded in the presence of (*R*)-2,2,2-trifluoro-1-(9-anthryl)ethanol, H-1' was observed as a singlet at δ 4.32 for (+)-**6a** and at δ 4.30 for (-)-**6b**. Only one resonance was observed for H-1' in the ^{1}H NMR spectrum of each enantiomer. Optical rotations in $CHCl_3$ at 27 °C also were recorded: (+)-**6a** had $[\alpha]_D$ = + 11.9° and (-)-**6b** had $[\alpha]_D$ = - 12.1°.
19. The initially assigned absolute configuration of diol (-)-**6b** was later verified using the method of Mosher[20] on the alcohol obtained by reduction of (-)-**13b**. See footnote 22.
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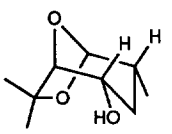
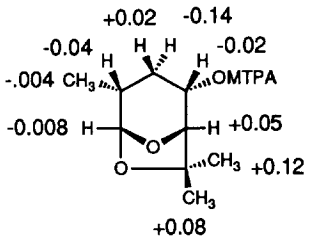
21. When the ^1H NMR spectra were recorded in the presence of (*R*)-2,2,2-trifluoro-1-(9-anthryl)ethanol, H-1 and H-5 were each observed as singlets at δ 5.22 and δ 3.86, respectively, for (+)-**13a** and at δ 5.23 and δ 3.87, respectively, for (-)-**13b**. Only one resonance was observed for H-1 and H-5 in the ^1H NMR spectrum of each enantiomer. Optical rotations in hexane at 26° C also were recorded: (+)-**13a** had $[\alpha]_D$ = + 6.8° and (-)-**13b** had $[\alpha]_D$ = - 7.0°.
22. Reduction of compound (-)-**13b** with K-Selectride® in CH_2Cl_2/THF gave as the only product (+)-**14b.** Treatment of this alcohol with (*S*)-(+)-α-methoxy-α-(trifluoromethylphenyl)acetyl chloride [(+)-MTPA-Cl] and pyridine in CH_2Cl_2 gave the (*R*)-MTPA ester whereas treatment with (*R*)-(-)-MTPA-Cl gave the (*S*)-MTPA ester. Analysis of the $\Delta\delta$ (δ_S - δ_R) from the ^1H NMR of the (*S*)- and (*R*)-MTPA esters indicates that (-)-**13b** has the absolute configuration shown in Scheme 2. This absolute configuration for (-)-**13b** is in agreement with what was predicted from the mnemonic used to assign the absolute configuration of the (-)-**6b** diol precursor.



(+)-**14b**



Δδ Values from analysis of MTPA esters.

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