cenes, and the chromatograms were divided into 8 bands. Bands 1, 2, 4, 5 and 7 correspond to the origin, nivalenol, neosolaniol and HT-2 toxin, fusarenon-X, and T_2 toxin and diacetoxyscirpenol, respectively. The silica gel from each band was scraped from the plates and eluted with acetone. The eluate was evaporated in a microtube under a stream of nitrogen gas; the residue was derivatized with 20 µl of a silylating reagent [N,O-bis (trimethylsilyl) acetamide - N-trimethylsilylimidazole-trimethylchlorosilane, 5:5:1, v/v/v], and 1 µl was subjected to GLC analysis. Zearalenone was only present in band 8 and was analyzed by GLC as in the case of trichothecenes. GLC analyses were carried out on a Schimadzu GC-4B gas chromatograph equipped with a flame ionization detector. A stainless steel column (0.3 × 100 cm) packed with 1.5% OV-1 on 100-120 mesh Chromosorb W was used. The column temperature was programmed from 180 °C to 280 °C at 5 °/min and the flow rates of carrier gas (nitrogen), hydrogen and air were 40, 35 and 800 ml/min, respectively. The temperatures of the injection port and the detector were set at 280 °C and 300 °C, respectively.

Results and discussion. The dermatic properties of the toxic compounds in the extracts have been used as indicator and basis for semi-quantiative analysis. E_2 and E_3 show positive response, necrosis is observed after 48 h. E_1 (control) has no effect. Analysis by GLC (table) showed the presence of nivalenol, deoxynivalenol and T_2 toxin in (E_2) and only the 2 former toxins in (E_3). Both extracts contained also zearalenone.

To our knowledge, only 2 publications describe the presence of nivalenol and deoxynivalenol as natural contaminants: Morooka et al.[3] mentioned deoxynivalenol and nivalenol in barley and Mirocha et al.[4] reported deoxynivalenol, T_2 toxin and diacetoxyscirpenol in mixed feedstuff. Deoxynivalenol was reported to be a feed-refusal and emetic factor[5], and it is likely that this toxin is involved in naturally occurring emesis and feed refusal in swine.

The presence of zearalenone is not unusual in that it has been commonly found as a natural contaminant[6] of corn often in mixtures with trichothecenes. It appears that among the trichothecenes compounds, deoxynivalenol, nivalenol and T_2 seem to be worldwide in distribution as natural contaminants.

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Stereoselectivity of oxotremorine antagonists containing a chiral pyrrolidine group

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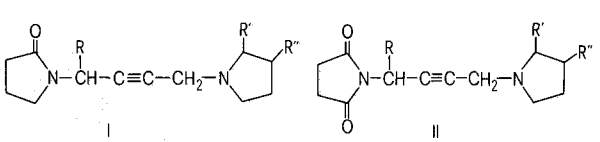
Summary. Oxotremorine **(Ia)** and its succinimide analogue **(IIa)** have been substituted in the pyrrolidine ring with a methyl group in the 2- or 3-positions. The compounds are oxotremorine antagonists. The 2-methyl-substituted enantiomers show stereoselectivity, the S-isomers being the most active.

In previous papers from our laboratories, it has been shown that introduction of a methyl group in the 1-position of the butynyl chain of the muscarinic agent oxotremorine, N-(4-pyrrolidino-2-butynyl)-2-pyrrolidone **(Ia)**, gives an antagonist **(Ib)** of high activity[1]. Similar substitution in the oxotremorine antagonist N-(4-pyrrolidino-2-butynyl) succinimide **(IIa)** affords an antagonist **(IIb)** which is considerably more active than the parent compound[2]. The enantiomers of **Ib** and **IIb** are highly stereospecific in blocking the motor effects of oxotremorine. The R-isomers were found to be about twice as active as their corresponding racemates, while the S-isomers were practically inactive[3].

In view of these results, we found it of interest to prepare oxotremorine analogues with a centre of chirality in the vicinity of the other polar group in the oxotremorine molecule, the basic nitrogen atom.



I II

	R	R′	R″
a	H	H	H
b	CH_3	H	H
c	H	CH_3	H
d	H	H	CH_3

Consequently, we synthesized chiral analogues of **Ia** and **IIa** with a methyl group in the 2- or 3-positions of the pyrrolidine ring **(Ic, Id, IIc, IId)**. The corresponding racemates were also prepared for comparative purposes. The compounds were prepared through the Mannich reaction from N-propargyl-2-pyrrolidone or N-propargylsuccinimide, formaldehyde and the appropriate methylpyrrolidine according to methods previously described[2]. The enantiomers of 2-methylpyrrolidine were obtained by resolution of the racemic amine using (+)- and (−)-tartaric acid[4]. The absolute configuration of the enantiomers has been established through correlation to L-proline[5,6]. The enantiomers of 3-methylpyrrolidine were prepared by reduction of the appropriate 3-methyl-2-pyrrolidone with $LiAlH_4$. (+)-3-Methyl-2-pyrrolidone was assigned the R configuration[7,8] but this was later questioned[9,10]. We therefore found it necessary to make a reinvestigation and we were able to establish the R configuration of (+)-3-methyl-2-pyrrolidone and of (+)-3-methylpyrrolidine by correlation to (R)-(+)-methylsuccinic acid[4].

The compounds were tested for their blocking action on the motor effects of oxotremorine and for mydriatic activity in intact mice at a standard dose of 20 µmoles/kg according to the screening methods described earlier[11].

The physical data for the new compounds and the results of the pharmacological tests are summarized in the table which also includes atropine as a reference compound.

Physical and pharmacological data for compounds **Ic**, **Id**, **IIc** and **IId**

Compound	Derivative	Melting point (°C)	$[\alpha]_D^{22}$*	In vivo dose (μmoles/kg) in mice required to produce oxotremorine blockade**	Mydriatic activity relative to atropine
(R)-(−)-Ic	Sesquioxalate	85–86	−22.9	56.0	0.06
(S)-(+)-Ic	Sesquioxalate	86–87	+22.9	2.6	0.07
(±)-Ic	Sesquioxalate	74–75		5.0	0.05
(R)-(+)-Id	Sesquioxalate	82–83	+ 1.2	6.5	0.024
(S)-(−)-Id	Sesquioxalate	82–83	− 1.4	5.8	0.017
(±)-Id	Sesquioxalate	91.5–92.5		5.2	0.024
(R)-(−)-IIc	Base	53.5–54.5	−58.4	17.4	0.024
(S)-(+)-IIc	Base	54.5–55.5	+59.9	8.3	0.027
(±)-IIc	Base	52–53		13.5	0.022
(R)-(+)-IId	Oxalate	104.5–106	+ 0.8	20.0	0.001
(S)-(−)-IId	Oxalate	105–106	− 0.8	17.2	Miosis
(±)-IId	Oxalate	108–110		17.9	Inactive
Atropine				2.8	1

*All rotations were measured in ethanol (c 1.0–1.7). **Dose of test compound required to double the dose of oxotremorine inducing a grade 2 tremor in 50% of the mice.

It is evident from the table that introduction of a methyl group in the pyrrolidine ring of oxotremorine **(Ia)** affords compounds which are antagonists to oxotremorine. The same substitution in the oxotremorine antagonist **IIa** enhances the antagonistic activity. Only the enantiomers substituted in the 2-position show stereoselectivity, the S-isomers being the most active. However, this selectivity is less pronounced than that of the enantiomers of the compounds with the chiral centre in the 1-position of the butynyl chain **(Ib, IIb)**[3]. These results are somewhat similar to those obtained with parasympatholytic agents of the amino ester type, whose activity is critically dependent on the configuration of a chiral centre in the acyl moiety but independent of the configuration of the amino alcohol part of the molecule[12]. The compounds in the table have weak mydriatic activity and no stereoselectivity can be observed.

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Renal accumulation of amikacin, tobramycin and gentamycin in the rat

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Summary. Free and total concentrations of amikacin, tobramycin and gentamycin were measured separately in the rat kidney after equal weight by weight doses. The accumulation of aminoglycosides followed the order amikacin < tobramycin < gentamycin. The ratio between free and total aminoglycosides was similar (about 0.6) in all 3 aminoglycosides and independent on the length of administration.

The aminoglycoside antibiotics are potentially nephrotoxic agents[1,2]. The renal accumulation of many aminoglycosides has been compared using mainly the rat as experimental animal[3–5]. However, the renal accumulation of amikacin, a new aminoglycoside derivative, in comparison with tobramycin and gentamycin, has not been established. In the present study, the renal accumulation of amikacin, tobramycin and gentamycin were investigated in young female rats. Since aminoglycosides become partially bound to tissue macromolecules and thus inactivated[6], free and total aminoglycoside concentrations were measured separately.

Material and methods. 1-month-old female Sprague-Dawley rats (90–110 g) were selected for the experiment. The rats were maintained on a standard diet (R3/Astra-Ewos) and tap water ad libitum. Amikacin (Bristol Laboratories), tobramycin (Nebcina®, Lilly) and gentamycin (Garamycin®, Schering) were administered at the dose of 40 mg/kg s.c. once a day. The treatment lasted 1, 5 or 12 days. The