maintenance of oxygen tension. Accumulation of carbon dioxide and water vapour was avoided. Enflurane, 3200 ppm (v/v) in air, i.e. 0.2 MAC, was added to the experimental group and the concentration in the cages monitored regularly by gas chromatography. The animals were given food and water ad libitum when not in the exposure chambers. The animals were killed just prior to delivery on the morning of day 22. The number of resorptions and dead foetuses and the sex of the offspring were noted. All foetuses were weighed and examined for external defects. Selected foetuses (control 10; experimental 12) were cleared and the skeletal system stained with Alizarin Red-S. The remaining foetuses (control 57; experimental 75) were fixed in Bouin's solution and examined later for visceral abnormalities by the Wilson's technique. Maternal liver, kidney and foetal liver were processed routinely for histological studies.

Results and discussion. A summary of our findings is presented in the table. There were no maternal deaths and all animals were found to be pregnant at the time of post-mortem. There were no significant differences in weight gain during the experimental period between treated and control animals. Exposure of the mothers to the anaesthetic agent did not significantly increase the incidence of foetal resorptions. All foetuses recovered at term were alive and showed no external defects. Major skeletal defects were not present in any of the alizarin-stained foetuses. There was, however, a significant decrease in the mean b.wt of foetuses of enflurane-exposed mothers when compared to the control. Microscopic examination of the maternal and foetal tissues revealed no pathological changes.

This preliminary investigation indicates that as with nitrous oxide[12], halothane[11,12] and methoxyflurane[12], high subanaesthetic concentrations of the inhalation agent enflurane may cause foetal growth retardation without any significant foetal loss or abnormalities. Pope et al.[12] have previously demonstrated that food deprivation alone, in the amounts caused by appetite suppression after chronic exposure to inhalation anaesthetics, does not cause intrauterine growth retardation. Thus, enflurane may be less harmful during pregnancy than might have been suspected from previous studies with other volatile anaesthetic agents.

1 A.I. Vaisman, Eksp. Khir. Anest. *3*, 44 (1967).
2 V. Askrog and B. Harvald, Saertr. Nord. med. *83*, 490 (1970).
3 R.P. Knill-Jones, D. Moir, L.V. Rodrigues and A.A. Spence, Lancet *1*, 1326 (1972).
4 Report of ASA Ad Hoc Committee: Occupational disease among operating room personnel. Anesthesiology *41*, 321 (1974).
5 B.R. Fink, T.H. Shepard and R.J. Blandau, Nature *214*, 146 (1967).
6 A.B. Basford and B.R. Fink, Anesthesiology *29*, 1167 (1968).
7 D.A. Bussard, R.K. Stoelting, C. Peterson and M. Ishaq, Anesthesiology *41*, 275 (1974).
8 R. Wittmann, A. Doenicke and H. Heinrich, Anaesthesist *23*, 30 (1974).
9 G.L. Kennedy, S.H. Smith, M.L. Keplinger and J.C. Calandra, Toxic. appl. Pharmac. *35*, 467 (1976).
10 W.D.B. Pope, M.J. Halsey, A.B.G. Lansdown, A. Simmonds and P.E. Bateman, Acta anaesth. belg. *26*, 169 (1975).
11 A.B.G. Lansdown, W.D.B. Pope, M.J. Halsey and P.E. Bateman, Teratology *13*, 299 (1976).
12 W.D.B. Pope, M.J. Halsey, A.B.G. Lansdown, A. Simmonds and P.E. Bateman, Anesthesiology *48*, 11 (1978).

Natural occurrence of trichothecenes (nivalenol, deoxynivalenol, T_2) and zearalenone in corn

M. Jemmali, Y. Ueno, K. Ishii, C. Frayssinet and M. Etienne

Service mycotoxines, I.N.R.A. 16, rue Nicolas Fortin, F-75013 Paris (France); Faculty of Pharmaceutical Sciences, Tokyo University of Science, Shinjuku-Ku, Tokyo 162 (Japan); I.R.S.C., F-94800 Villejuif (France), and Elevage porcs, I.N.R.A., F-78350 Jouy en Josas (France), 2 March 1978

Summary. Samples of corn suspected of causing infertility and refusal symptoms were analyzed and found to contain nivalenol, deoxynivalenol, T_2 toxin and zearalenone, metabolites from *Fusarium* species.

Several sporadic cases of mycotoxicosis were reported from animals consuming cereal grains, particularly corn. This cereal is often implicated in symptoms such as abortion, emesis, and feed refusal. *Fusarium* species have been shown to be causative agents in producing mycotoxins inducing these symptoms. Samples from 2 lots of corn in France suspected of causing infertility, hyperestrogenic signs and feed refusal in swine were analyzed for zearalenone and trichothecenes, using thin layer chromatography (TLC) and gas-liquid chromatography (GLC) and the biological rat skin test.

Experimental. Preparation of extract: The extracts (E_1, E_2, E_3) were prepared from samples according to the method described by Ueno et al.[1]. The methanolic solution was washed with hexane to remove corn lipids and the aqueous methanol was evaporated to dryness. The residue was extracted with methanol-chloroform (1:5). Only this latter soluble fraction was analyzed. The control extract (E_1) was prepared from freshly harvested corn.

Dermic test. The extracts were dissolved in acetone and applied topically to clipped skin rats according to the method described by Frayssinet[2]. Skin was inspected after 24, 48 and 72 h.

Chemical procedure. The extracts (E_1, E_2, E_3) were dissolved in methanol and the amount equivalent to 10 g of the original samples was applied on TLC plates of 0.5 mm thickness (20 × 20 cm). The standard solutions of nivalenol, neosolaniol, deoxynivalenol, fusarenon-X, and T_2 toxin were applied on both ends of the plates and developed with benzene-acetone (12:7, v/v). The edges of the plates were charred with H_2SO_4 to visualize the standard trichothe-

Concentration of toxins

	E_1	E_2	E_3
Zearalenone	ND	10 ppm	2.5 ppm
Nivalenol	ND	4.28 ppm	1.18 ppm
Deoxynivalenol	ND	0.6 ppm	0.14 ppm
T_2 toxin	ND	0.02 ppm	ND

ND = not detected.

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.

1 Y. Ueno, K. Ishii, N. Sato and K. Ohtsubo, Jap. J. exp. Med. *44*, 123 (1974).
2 C. Frayssinet, Ann. Nutr. Alim., in press (1977).
3 N. Morooka, T. Uratsuji, T. Yoshizana and H. Yamamoto. Jap. J. Food Hyg. *13*, 368 (1972).
4 C.J. Mirocha, S.V. Pathre, B. Schauerhamer and C.M. Christensen, Appl. environ. Microbiol. *32*, 553 (1976).
5 R.F. Vesonder, A. Ciegler, A.H. Jensen, W.K. Rohwedder and D. Weisleder, Appl. environ. Microbiol. *31*, 280 (1976).
6 M. Jemmali, Ann. Microbiol. Inst. Part. *124B*, 109 (1973).

Stereoselectivity of oxotremorine antagonists containing a chiral pyrrolidine group

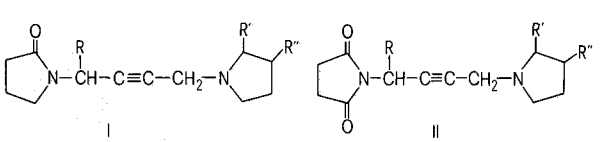
B. Ringdahl and R. Dahlbom

Department of Organic Pharmaceutical Chemistry, Biomedical Center, University of Uppsala, Box 574, S-751 23 Uppsala (Sweden), 13 March 1978

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.