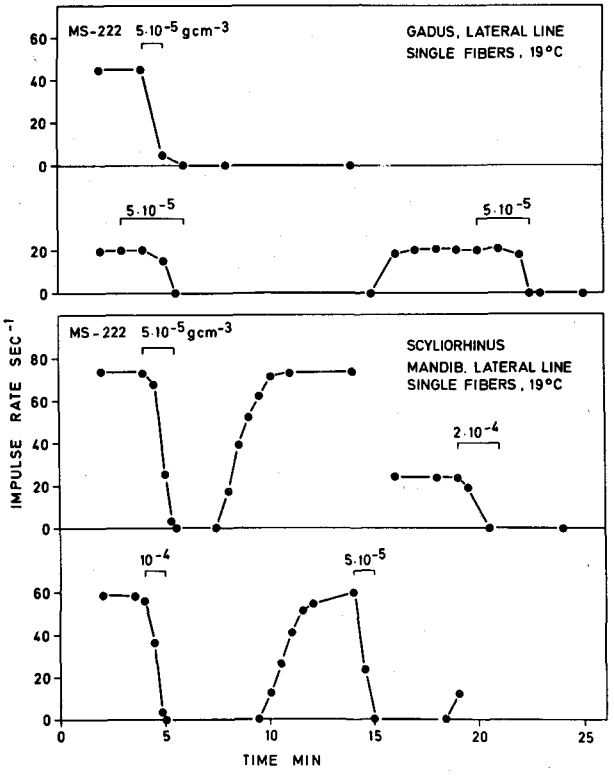

Fig. 3. Static impulse rate at 19 °C of various single fibres from lateral-line organs in *Gadus* and *Scyliorhinus* when applying MS-222. During the remaining time intervals, the preparation was bathed with saline.

NICA[6]). However, in these particular experiments, the disturbance by the anesthetic may possibly be neglected since the isolated lateral-line preparations had been bathed in saline for a longer period of time before impulses were recorded[7,8].

Zusammenfassung. Aethyl-m-Aminobenzoat (MS-222) führt in Konzentrationen von 10^{-3} bis $5 \cdot 10^{-5}$ g cm^{-3} an Lorenzinischen Ampullen zu einer kompletten und reversiblen Hemmung der Spontanaktivität, der dynamischen Reaktion bei Kältesprüngen und der elektrischen Reizantwort. Ebenso hemmt es die Spontanaktivität von Seitenlinienorganen. Diese periphere Wirkungskomponente der als Kaltblüter-Anaestheticum verwendeten Substanz muss bei allen Versuchen an peripheren Rezeptoren berücksichtigt werden.

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A Central Nervous System Depressant-Antidepressant

Tricyclic antidepressants of the imipramine class are accepted as useful in the treatment of endogenous (involutional) depressive illnesses. Instances of 'pure' involutional depression are in the minority and usually anxiety is associated with depression[1]. Such mixed depressed anxiety states are frequently treated with antidepressants producing marked sedative effects, viz., amitriptyline or doxepin[2] or combinations of an antidepressant and a neuroleptic[3] or anxiolytic[4]. Anxiolytic or even sedative-hypnotic therapy, viz., diazepam or phenobarbital alone is also used[2,5,6].

The present report deals with a new tricyclic agent distinguished by the presence of an allenic side chain. It possesses properties which may be ideally suited for mixed anxiety-depressive therapy. This agent, 5-(3-dimethylaminoprop-1-enylidene)-5H-dibenzo [a, d] cycloheptene maleic acid salt, abbreviated DMPD (IVb), exerts marked antidepressant effects and simultaneously is a potent central nervous system (CNS) depressant.

Methods. Reversal of reserpine (RES) induced hypothermia[7] was used to assess the antidepressant action of DMPD and other agents. Albino mice (18–24 g) were given RES (5 mg/kg, i.p.). 2 h later test material was administered orally. 1 h later and at hourly intervals for 4 h, rectal temperatures were recorded. Percent reversal of hypothermia = (D-R/C-R) 100, where D, R and C refer to mean rectal temperatures of drug treated reserpinized mice, reserpinized untreated mice and control animals, respectively.

DMPD was given i.p. to mice at a dose of 10 mg/kg 30 min after treatment with the MAO inhibitor pargyline (30 mg/kg, p.o.). L-DOPA was given $4^1/_2$ h later to these mice. The degree of CNS excitation evoked in such mice was compared to untreated mice or mice receiving either pargyline and L-DOPA or L-DOPA alone.

Behavioral changes produced in normal mice by compounds under study were also systematically evaluated[8]. Large groups of mice were used to obtain ED_{50} estimates[9,10] of each agent's ability to produce a loss of righting for a 30 sec period.

The effect of DMPD on blood pressure, heart rate and/or nictitating membrane responses was evaluated in 10 pentobarbital-anesthetized cats. In these preparations, alteration of various neurotransmitter and autocoid responses were measured.

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Results and discussion. DMPD displayed marked antidepressant effects. It was 4 to 16 times more potent than imipramine and slightly more potent than amitriptyline in reversing RES-induced hypothermia. Doxepin was only marginally active even when given at high dose levels (Table I). DMPD or known antidepressants did not produce hyperthermia in normal mice as do CNS stimulants.

Like imipramine, DMPD augmented the excitant effects of pargyline and L-DOPA in mice. DMPD did not increase brain concentrations of norepinephrine or dopamine in such mice. EVERETT[11] observed a similar lack of activity with imipramine.

Behavioral studies carried out in normal mice revealed that DMPD is a potent CNS depressant. Dose levels as low as 10 mg/kg, i.p. produced abnormal postural changes resulting in wobbly gait, disorientation and ataxia, and at 70 mg/kg upward a loss of righting ability was seen. Imipramine and amitriptyline induce mixed stimulant-depressive conditions in mice but no loss of righting at non-lethal dose levels, while doxepin, like DMPD, was found to be a highly potent CNS depressant. DMPD produced primarily depression; tremor and convulsive seizures were seen at high toxic dose levels. DMPD and other CNS depressants were studied in detail for their ability to produce a loss of righting in mice and Table II summarizes these studies. DMPD appears to be definitely a more potent CNS depressant than meprobamate and probably is more potent than phenobarbital and chlordiazepoxide but not doxepin, diazepam or chlorpromazine. The intraperitoneal LD_{50} of DMPD derived from animals dying acutely and directly as a consequence of CNS depression was 107 (102–112) mg/kg. However, in common with tricyclic antidepressants, additional lethalities occurred after recovery from CNS depression. The LD_{50} including delayed deaths was 94 (86–103) mg/kg.

Like other tricyclic antidepressants, DMPD produces anticholinergic effects in mice and cats and norepinephrine augmenting, α-adrenolytic and antihistaminic activity in anesthetized cats.

A compound producing such a mixture of potent antidepressant-depressant activity as shown in our animal models potentially may be of great clinical usefulness.

The synthesis of DMPD was effected as shown in the Formulae. The acetylenic Grignard reagent (II)[12], prepared in situ from vinyl magnesium chloride and 3-N,N-dimethylaminoprop-1-yne, was reacted at 50 °C in tetrahydrofuran with 5-chloro-5H-dibenzo [a, d] cycloheptene (I)[13] to furnish 5-(3-N,N-dimethylaminoprop-1-ynyl)-5H-dibenzo [a, d] cycloheptene (IIIa)[14]. Treatment of an ethereal solution of (IIIa) with ethereal maleic acid furnished the crystalline maleic acid salt (IIIb) [mp 158.5–160][14]. When an ethereal solution of (IIIa) was stirred for 1 h at room temperature with alkaline alumina (WOELM, Activity I), quantitative rearrangement to the oily allenic amine (IVa) [ν_{max}^{film} 1930 cm^{-1}][14] was effected. Treatment of (IVa) with maleic acid in an

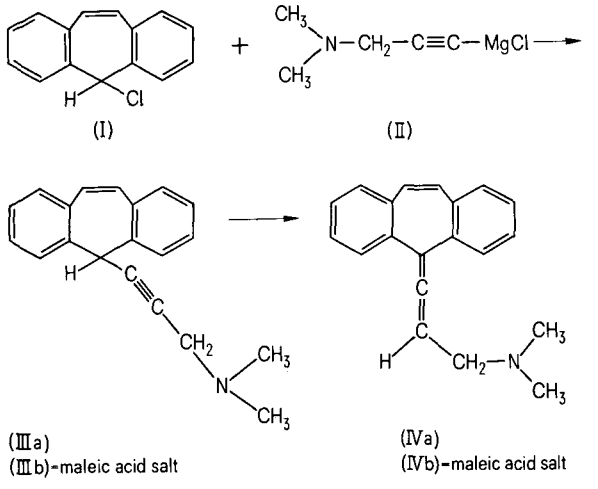

Table I. Effect of various known antidepressants and DMPD in reversing reserpine-induced hypothermia in mice

Drug	Dose (mg/kg, p.o.)	Reserpine hypothermia reversal (%) 1 h	2 h	3 h	4 h
Imipramine	10	36[a]	33[a]	39[a]	21[a]
DMPD		95[a]	68[a]	66[a]	47[a]
Imipramine	2.5	10	1	16[b]	10
DMPD		34[a]	34[a]	28[a]	24[a]
Imipramine	0.62	26	18[b]	15	2
DMPD		40[a]	31[a]	27[a]	29[a]
Imipramine	0.15	13	0	0	0
DMPD		15	27	20	14
Amitriptyline	10	38[b]	44[a]	47[a]	48[a]
DMPD		52[a]	46[a]	55[a]	60[a]
Amitriptyline	2.5	23	22	30	33[a]
DMPD		51[a]	31[a]	36[a]	44[a]
Amitriptyline	0.62	28	17	11	0
DMPD		13	10	19[b]	12
Amitriptyline	0.15	7	0	0	0
DMPD		20	2	6	14
Doxepin	40	71[b]	32[a]	23[a]	28[a]
	10	15	9	0	0
	2.5	14	0	0	0
	0.62	0	0	0	0

[a] Differs significantly (analysis of variance) from reserpine control at probability level of $p < 0.01$. [b] $p < 0.05$.

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[14] IR, NMR and mass spectral data consistent with the assigned structures, as well as satisfactory elemental analyses in the case of (IIIb) and (IVb), were obtained for these compounds.

Table II. Comparative CNS depressant potency and toxicity of DMPD and various drugs given i.p.

Compound	Loss of righting ED_{50} (mg/kg)	LD_{50} (mg/kg)	LD_{50}/ED_{50}
DMPD	82 (76–89)[a]	107 (102–112)[a]	1.3
Doxepin	40 (37–43)	50 (33–75)	1.3
Imipramine	No righting loss	85 (70–99)	–
Amitriptyline	No righting loss	75 (52–101)	–
Meprobamate	212 (191–228)	467 (366–595)	2.2
Phenobarbital	103 (72–114)	222 (188–343)	2.2
Chlordiazepoxide	89 (61–107)	227 (202–260)	2.6
Diazepam	28 (22–34)	321 (206–502)	11.5
Chlorpromazine	12 (7–16)	85 (77–153)	7.1

[a] 95% Confidence limits shown in parentheses.

analogous manner to that described for (IIIa) then furnished (IVb) (DMPD) as a crystalline maleic acid salt [mp 131–132][14].

[15] We wish to thank Abbott Labs. for sample of pargyline; Geigy Pharmaceutical Co. for imipramine; Hoffmann-La Roche, Inc. for chlordiazepoxide and diazepam; Merck Sharp and Dohme for amitriptyline; Pfizer Labs. for doxepin; and Smith Kline and French Labs. for chlorpromazine.
[16] The authors wish to express their gratitude to DAGMAR J. KUNC for her expert technical assistance.
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Summary. A new tricyclic agent with an allenyl side chain experimentally shows antidepressant activity similar to amitriptyline and imipramine but also exerts marked CNS depression. Such dual activity should be of clinical interest for treatment of mixed anxiety and depression.

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Inhibition of Drug Metabolizing Enzymes by Diazepam in Rat Liver

Hypnotics and sedatives are known to stimulate or inhibit the drug-metabolizing enzymes in liver[1,2]. Benzodiazepines are widely used and are frequently prescribed in combination with other drugs. Chlordiazepoxide has been suggested to be an enzyme inducer[3–6]. In one study[7] it was shown that diazepam decreased the pentobarbital sleeping time and serum concentration, but the inducing effect could not be confirmed in another study[6]. Chlordiazepoxide has been shown to stimulate its own metabolism in rat[3], and a tolerance to diazepam has been described in cat[8]. Ethanol seems to enhance the action of diazepam[9] but the mechanisms of these effects of diazepam are not known. We investigated microsomal metabolism of hexobarbital, N-methylaniline and *p*-nitrobenzoic acid as well as the content of cytochrome P-450 in rat liver after diazepam treatment. Further we studied whether diazepam can stimulate its own metabolism and thus explain the developing tolerance. We also compared diazepam to two well-known stimulators of microsomal metabolism, phenobarbital and 3,4-benzpyrene.

Materials and methods. 4 groups of 6 male Sprague-Dawley rats weighing about 200 g were kept on a standard diet. 1 group (controls) received vehicle solvent, 2nd group diazepam 100 mg, 3rd group phenobarbital 80 mg and 4th group 3,4-benzpyrene 20 mg/kg of body weight. All drugs were given by mouth once a day for 6 days in a vehicle solvent consisting of tween 20 and carboxymethyl cellulose (1:4).

Preparation of livers. Animals were decapitated 24 h after the last dose. The livers were removed and rinsed with ice-cold 0.1 *M* phosphate buffer (pH 7.4). All subsequent manipulations were carried out at 2–4 °C. The 20% liver homogenates were prepared in the same phosphate buffer with a Potter-Elvehjem type homogenizer. The homogenate was centrifuged at 12,000 *g* for 20 min. Part of the supernatant was then centrifuged at 105,000 *g* for 1 h. The microsomal pellet was suspended in the phosphate buffer so that 2.5 ml suspension corresponded to 1 g of liver tissue.

Enzyme assays. The 12,000 *g* supernatant was used to assay the hexobarbital hydroxylation, *p*-nitrobenzoic acid reduction, N-methylaniline demethylation and diazepam metabolism. The microsomal suspension was used to measure the cytochrome P-450 content. The incubation conditions for assays of the metabolism of hexobarbital, N-methylaniline and *p*-nitrobenzoic acid have been described by VORNE and ARVELA [10], and that of diazepam by SCHWARTZ and POSTMA[11]. The amounts of the sub-

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Table I. Effect of diazepam (DZP), phenobarbital (PB) and 3,4-benzpyrene (BP) treatment on cytochrome P-450 content and the rates of metabolism of hexobarbital, *p*-nitrobenzoic acid and N-methylaniline in rat liver microsomes

Group	Cytochrome P-450 content (nmol/g liver)	(%)	Rate of metabolism of substrates (nmol/g liver/h)					
			Hexobarbital	(%)	*p*-Nitrobenzoic acid	(%)	N-methylaniline	(%)
Control	8.3 ± 1.6	100	6.95 ± 0.70	100	2.51 ± 0.72	100	1.57 ± 0.29	100
DZP	7.5 ± 0.8	90	4.60 ± 0.93[b]	67	1.49 ± 0.23[a]	59	1.50 ± 0.34	95
PB	19.1 ± 3.4[b]	230	11.10 ± 0.67[b]	160	5.79 ± 2.30[a]	230	4.49 ± 0.58[b]	285
BP	10.6 ± 3.6	128	5.07 ± 1.15[a]	73	2.44 ± 0.43	97	1.43 ± 0.34	91

Mean value ± SD of 6 rats. [a] $p < 0.01$; [b] $p < 0.001$.