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Anaesthetic Action and Toxicity of Oxetanes in White Rats

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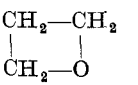
The well known anaesthetic properties of *cyclo*propane and of ethers suggested to Krantz and co-workers[1] the possibility of combining these structural features to make an improved anaesthetic. The resulting *cyclo*propyl methyl ether, *cyclo*propyl ethyl ether[2] and *cyclo*propyl vinyl ether[3] all proved to be potent anaesthetics. *cyclo*Butane is also an effective anaesthetic,[4,5] though recently it has been reported inferior to *cyclo*propane.[6] No *cyclo*butyl ethers seem to have been tested.

If the anaesthetic action of *cyclo*propane and *cyclo*butane is due to the unique properties of three- and four-membered rings, it might be possible to enhance the action by inserting an ether function within the ring. It is indeed found that ethylene oxide, the cyclic ether analogue of *cyclo*propane, has anaesthetic action comparable to ethyl ether, but is quite toxic. The toxicity is not surprising in view of its reactivity with protein[8] and with water, the latter forming the highly toxic ethylene glycol.

The cyclic ether analogue of *cyclo*butane, trimethylene oxide or oxetane (I), however, is known to hydrolyse very slowly (except



(I)

under strongly acid conditions)[9] and its hydrolysis product, 1,3-propanediol, appears to be practically non-toxic. Furthermore, oxetane probably could not react with proteins since it does not react with amino groups[10] and in general shows a much lower order of reactivity than ethylene oxides. The physical properties of oxetane appeared to be compatible with possible anaesthetic

action; it is a volatile, colourless, mobile liquid with a pleasant, non-irritating odour and a fairly high water solubility.

In this work oxetane was administered to white rats subcutaneously, intraperitoneally and orally, as well as by inhalation. The study was extended to a number of methyl- and ethyl-substituted oxetanes which were available in these laboratories and which had physical properties resembling those of the parent ether, except for lower vapour pressure and water solubility. Special interest attaches to one of these, 3,3-diethyloxetane, because it has the same carbon skeleton as the excellent anticonvulsant, Prenderol.[12]

Experimental

Materials. The oxetanes studied are listed in Table I, along with references to the methods of preparation used. The compounds were purified by fractional distillation, the centre cuts boiling within 1° being used. The b.p., refractive indices, and densities that had been previously reported were in good agreement with the literature values.

Table I. Properties of oxetanes

Compound	b.p., °	d_4^{20}	Mol. wt.	Ref.
Oxetane	47	0·873	58	11a
2-Methyloxetane	61–62	0·836	72	*
2,2-Dimethyloxetane	70	0·819	86	†
3,3-Dimethyloxetane	77	0·846	86	‡ §
3-Ethyloxetane	95–96	0·850	86	‖
3,3-Diethyloxetane	129	0·849	114	§ ‖

* Searles, S., Pollart, K. A. and Block, F. *J. Amer. chem. Soc.* **79**, 952 (1957) †Bennett, G. and Philip, W. *J. chem. Soc.* **1928**, 1930 ‡ Searles, S. and Gortatowski, M. J. *J. Amer. chem. Soc.* **75**, 3030 (1953) § Searles, S., Hummel, D. J., Throckmorton, P. E., Nukina, S. and Hays, H. *J. Amer. chem. Soc.* (submitted) ‖ Searles, S., Nickerson, R. G. and Witsiepe, W. K. *J. org. Chem.* (submitted)

The vapour pressure of oxetane was determined by means of a nitrometer as about 250 mm at 25°. The solubility in water at 25° was found to be approximately 26 per cent, v/v, by the following method: A sealed ampoule containing 3 ml of oxetane

and 10 ml of water was shaken in a thermostated water bath for 10 h; after this treatment the volume of the oxetane layer amounted to 0·4 ml.*

Methods. Subcutaneous and intraperitoneal injections were made with a 24 gauge needle on a tuberculin syringe. Oral administration was accomplished by use of a long cannula direct into the stomach. An ether cone was used for inhalation, and a minimal amount of oxetane was dripped on a gauze as is customary with ether administration.

Reactions to oxetane administration were recorded on the basis of degree of anaesthesia: *Stage 1*—increased rate of respiration, relaxation of abdominal muscles, and grogginess; *Stage 2*—loss of locomotor control but continued excitability; *Stage 3*—all muscular reactivity, including toe and eye reflexes, were lost and the animal was in 'surgical anaesthesia'. Animals that recovered were kept under observation for five days, if they lived that long. Necropsies were carried out soon after death.

Results

The data from typical experiments are recorded in Tables II and III; most of the data were checked by running duplicate or closely similar experiments.

Respiratory administration of oxetane produced rapid and complete anaesthesia, as shown in Table II, but if maintained for more than 3 min invariably resulted in death within a few hours. Recovery from the anaesthesia was rapid and complete if the ether cone was removed as soon as stage 3 had been achieved, but after a few min of such anaesthesia, recovery was not complete. Righting and crawling ability returned rapidly, but ability to walk never returned, and in 2 h the animal became progressively weaker and died from respiratory failure. Post-mortem examination revealed capillary haemorrhage in the lungs and on the surface of the brain.

Oral administration, up to 10 g/kg, had no visible effects other than increased salivation.

* The previous report that oxetane is miscible with water (Derick, C. and Bissell, D., *J. Amer. chem. Soc.* **38**, 2485 (1916)) is not supported by any data and is apparently in error.

Table II. Anaesthetic and toxic effects of oxetane on rats.*

Method of administration	Rat wt., g	Dose		Min* to reach anaesthetic stage†						Death, h
		ml	g/kg	1	2	3	2	1	0	
Respiratory	100			1	2	3	4	5	20	
	110			1	2	3	6	8		3
	180			1	1·5	2	12	15		2·5
Oral	55	0·2	3·6				no anaesthetic effects			
	50	0·3	6·0							
	50	0·5	10·0							
Subcutaneous	80	0·3	3·8	3	4		15	60	120	
	90	0·4	4·5	3	4		20	110	180	
	160	0·8	5·0	4	6					4·3
Intraperitoneal	400	0·1	0·25	2						41
	400	0·18	0·45	2						24
	400	0·3	0·75	1	1·5	2	25			4
	185	0·6	3·2	1·5	3	4	45			1
	55	0·6	11·0		0·5	1				0·1
I.P., in olive oil	350	0·25	0·70	5	10		20	40	180	
	340	0·5	1·5	3	6		25	60	240	
	330	1·0	3·0	1	2	4	40	200		18
I.P., in 25% ethyl alcohol	400	‡		8				20	35	
	400	0·1	0·25	5	8		25	35	120	40
	400	0·2	0·50	3	5	8				4

* Time from beginning of administration to the onset of the designated stage is indicated. Generally the test animal passed successively through stages 1, 2, and 3, then reversed through 2 and 1, and possibly to full recovery, designated as 0.

† Symptoms of stages were considered to be: stage (1) animal became somewhat groggy, abdominal muscles relaxed, hind legs uncontrollable; stage (2) animal was excitable but had no control, continuing until it was no longer able to right itself or give eye lid reflex; stage (3) surgical anaesthesia with no reflexes or at most a slight twitch when the foot pad was pinched. ‡ Intraperitoneal injection of 2·5 ml of 25% ethyl alcohol.

Subcutaneous administration (Table II) produced a slow reaction, resulting in muscular relaxation, loss of control of the hind legs, then gradual weakening and death following the larger doses used. However, with the smaller doses, the animal gradually recovered, but the skin around the site of injection became necrotic and sloughed in about three days.

Intraperitoneal administration produced rapid and consistent results. Dosages less than 0·2 g/kg produced no visible effects other than irregular, jerky breathing; but effects became increasingly severe in a shorter time as the dosage was increased. Dosages of 0·25 g/kg of pure oxetane administered intraperitoneally invariably resulted in the death of the animal with bloody exudate from capillary rupture at the site of the injection and capillary rupture on the surface of the brain. There were regularly 5 to 15 haemorrhages over the surface of the cerebrum and more along the brain stem.

Dilution of oxetane with an equal volume of olive oil greatly moderated the effects on intraperitoneal injection. The action was considerably slowed, and amounts tolerated without causing deep anaesthesia or death were at least ten times greater. Sublethal doses, however, caused such severe irritation of the peritoneum that adhesions invariably resulted. Quantities sufficient to cause anaesthesia proved to be lethal even when diluted with olive oil.

With one exception, the substituted oxetanes used were similar in their physiological effects to oxetane itself, as they were quite toxic in the large doses required to bring about anaesthesia (Table III). However, 3,3-diethyloxetane was effective in about 20 per cent of the quantity required of the others. Injected undiluted, it was extremely toxic, rapidly producing local and brain haemorrhage of lethal proportions in doses as low as 0·5 g/kg. Diluted 1 : 5 with olive oil, however, it was relatively non-irritating and produced complete surgical anaesthesia in doses as low as 0·4 g/kg, followed by uneventful recovery. The olive oil–diethyloxetane mixture placed in the stomach was approximately half as effective as when injected intraperitoneally. Dilution with ethyl alcohol, on the other hand, greatly enhanced the effectiveness as well as the toxicity of the oxetane, indicating synergistic action.

In each case, the characteristic odour of the oxetanes appeared

Table III. Anaesthetic and toxic effects of substituted oxetanes on rats.

Substituent	Rat wt., g	Dose*		Min. to reach anaesthetic stage†						Death, min
		ml	g/kg	1	2	3	2	1	0	
2-Methyl	130	0·2	1·5	16	30					380
	335	1·0	3·0	1·5	3	35	125			240
	95	0·55	6·0	1	2	4				80
	110	0·8	7·5	1	2	3				7
2,2-Dimethyl	120	0·3	2·5	2	4	7	15			155
	55	0·3	5·0	1	2	3				70
3,3-Dimethyl	100	0·2	2·0	2	3	4	20			35
	100	0·3	3·0	1	1·5	2				40
	60	0·3	5·0							0·5
2,2-Diethyl	120	0·3	2·5	8	12	18				60
	60	0·2	5·0	4	6	10				40
	60	0·3	5·0	1	1·5	2				3
3,3-Diethyl	350	0·035‡	0·1	12				40	60	
	360	0·072‡	0·2	10				60	90	
	360	0·144‡	0·4	2	3	7	40	75	160	
	85	0·05	0·6	2	3	8	15			22
	100	0·1	1·0	1	2	4				8
	350	0·07§	0·2	40				90	120	
	350	0·132§	0·4	10	35		50	150	160	
	350	0·264§	0·8	5	8	12	20	80	240	

* Intraperitoneal administration unless otherwise specified. † As defined in footnotes (*) and (†), Table II. ‡ Diluted 1 : 4 with olive oil.
§ Diluted 1 : 4 with olive oil, placed in the stomach with a stomach tube.

strongly in the breath of the rats within a few min after injection and persisted for at least 1 h—several in the case of 3,3-diethyloxetane—after recovery. It seems likely that the mode of elimination was largely by respiration.

Discussion

This seems to be the first study of the pharmacological effects of oxetanes. The results show that they possess potent anaesthetic properties although the toxic effects of all the oxetanes studied here, except 3,3-diethyloxetane, may be too great to permit their practical application as anaesthetics. These may be overcome in the future, however, by further studies on methods of administration, perhaps with other substances, and on other substituted oxetanes.

The toxic properties seem to be associated with rupture of capillaries in the brain, resulting in respiratory failure. As blood pressure and other physiological reactions were not measured, the cause of capillary rupture is not certain, but it seems likely that it was due to an irritating or corrosive action on the capillary wall, since a similar condition was observed at the site of injection. The lowering of toxicity by dilution with olive oil may be due to reduction of concentrations of oxetanes in contact with the tissues.

The rapid induction would suggest ability to penetrate the nerve cells rapidly. The relative slowness in achieving complete recovery would suggest that the body cannot metabolize these substances or eliminate them except through the lungs.

The lethal dose of oxetane in olive oil can be estimated as over 2 ml/kg for rats, administered intraperitoneally, with the oral dose much higher. The toxicity is thus comparable to ethyl ether and other substances of anaesthetic value.[13] The introduction of the ethyl group in 3,3-diethyloxetane increases the toxicity somewhat but increases the effect on the central nervous system so much that much smaller doses can be used. The lethal dose of this compound in olive oil is over 0·4 ml/kg intraperitoneally and over 0·8 ml/kg orally. It is apparently no more toxic than the closely related anticonvulsant drug, 2,2-diethyl-1,3-propanediol (Prenderol), which has an LD_{50} of 0·85 g/kg.[14]

There has been some effort to prolong the effect of 2,2-diethyl-1,3-propanediol by converting it to various derivatives, from

which it may be slowly released in the body.[15] One might think that 3,3-diethyloxetane would be in this category, for it would undoubtedly hydrolyse slowly to 2,2-diethyl-1,3-propanediol. The observed action appears to be due to the oxetane itself, however, rather than to 2,2-diethyl-1,3-propanediol formed by hydrolysis, for the following reasons: (1) Hydrolysis is extremely slow,[11e,f] while the physiological action is rapid.* (2) The action of 3,3-diethyloxetane is somewhat different from that of 2,2-diethyl-1,3-propanediol. We are indebted to the Eli Lilly Co. for carrying out tests which showed anticonvulsant activity in pentylenetetrazol as well as electroshock seizures in rats with oral doses of 125 mg/kg and 293 mg/kg, respectively.[15] Prenderol gave no protection against pentylenetetrazol seizures. A somewhat greater dose of 3,3-diethyloxetane was required for electroshock seizures than of Prenderol but the effect was definitely longer-acting.[16]

Summary. Oxetane and several methyl- and ethyl-substituted oxetanes, administered to laboratory rats, showed anaesthetic activity, accompanied by considerable toxicity evidenced by capillary rupture. One of the compounds, 3,3-diethyloxetane, showed good anaesthetic, sedative and anticonvulsant effects when administered in olive oil in sub-lethal doses.

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* Also, the oxetane itself persists for several hours, as attested by the odour of the animal's breath, 2,2-diethyl-1,3-propanediol being virtually odourless.

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