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Some studies on cytisine and its methylated derivatives

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1. In mice cytisine hydrochloride is less toxic intravenously than nicotine hydrogen tartrate, but more toxic by intraperitoneal or oral administration. Compared with cytisine, caulophylline hydrogen iodide is one-fifth to one-tenth as toxic and caulophylline methiodide is less than one-thirtieth as toxic.

2. The surprising low oral toxicity of cytisine and nicotine may be ascribed to the method of administration; if the drug is placed directly in the stomach there is no possibility of absorption through buccal mucous membranes.

3. The peripheral effects of nicotine, cytisine and caulophylline are similar, though on some preparations those of nicotine last longer. In most tests cytisine is active in doses from a quarter to three-quarters of those of nicotine, caulophylline in doses from 10 to 20 times those of cytisine. Caulophylline methiodide is virtually inactive.

4. Cytisine and caulophylline may differ from nicotine in their central effects.

5. Cytisine and caulophylline are active as the cations. The pKa of cytisine is 7.92 and that of caulophylline is 7.04; the difference accounts, in part, for the weaker activity of caulophylline. The caulophylline ion is generally one-sixth to one-third as active as the cytisine ion.

6. The introduction of the second methyl group to form the quaternary salt does not appear to cause a dramatic change in the conformation of the molecule. Caulophylline methiodide appears to be feebly active because it has feeble affinity.

The alkaloid cytisine occurs in a number of plants of the leguminosae family and was considered by Dale & Laidlaw (1912) to be the toxic principle of the common laburnum. After tests in cats, rabbits and fowls, they described its peripheral actions as being " qualitatively indistinguishable from nicotine " though they observed that it differed from nicotine in that it did not produce an ear-twitch in cats. Zachowski (1938) confirmed its pharmacological resemblance to nicotine but, from experiments on the blood pressure of the cat, concluded that it had greater activity in stimulating sympathetic ganglia than in blocking them.

The chemical structure of cytisine was worked out by Ing (1931, 1932) and its absolute configuration is now known (Okudu & Katauku, 1961). It contains a

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secondary amino group (Fig. 1) and the tertiary base, N-methyl cytisine, occurs as the alkaloid caulophylline, together with cytisine, in several plants. The toxicity of caulophylline was examined by Kalaschnikow & Kusnetzow (1938) and its pharmacological properties briefly reported by Scott & Chen (1943). Although its peripheral actions resembled those of nicotine, the convulsions produced by caulophylline in mice differed from those produced by nicotine. The quaternary compound, caulophylline methiodide, was prepared by Ing (1931) but does not seem to have been tested pharmacologically.

This paper describes a comparison of the pharmacological properties of nicotine, cytisine, caulophylline and caulophylline methiodide. These compounds form an interesting chemical series because cytisine is a relatively rigid structure, and consequently the structural effects of methylation are limited to one particular part and unlikely to lead to changes in conformation elsewhere. The work also includes a study of the effects of changes in pH on the activity of cytisine and caulophylline on the frog rectus, to see whether these are active as the ions or as the uncharged base.

Methods

Chemical

Melting points were determined with a Mettler FP 1 instrument; analyses for halide are gravimetric with samples of 50–100 mg.

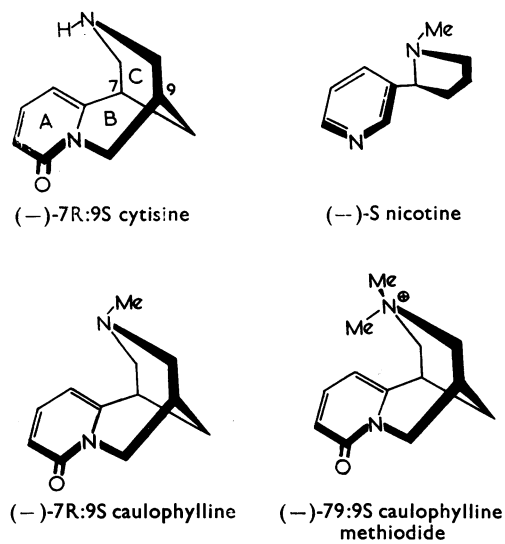

FIG. 1. Absolute configurations of (−)-cytisine, (−)-caulophylline and (−)-caulophylline methiodide (Okudu & Katauku, 1961) compared with (−)-nicotine (Hudson & Neuberger, 1950). Note the hydrogen atom attached to the basic nitrogen in ring C of cytisine can be in either of two conformations, but the methyl group attached to this atom in caulophylline is more likely to be as shown, than in the alternative arrangement (shown for the hydrogen atom in cytisine).

Cytisine melting point 152° C, $[\alpha]_{20^\circ}^{D}$ −119° in water, was bought from Fluka A.G.

Caulophylline hydrogen iodide was prepared by the method of Ing (1931) but with ethanol instead of methanol as solvent. Recrystallized material had m.p. 276.5° C ; found ; I^-, 38.08 ; calculated for $C_{12}H_{17}ON_2I$; I^-, 38.30%. Partheil (1892) recorded m.p. 270° C ; Ing (1931) recorded m.p. 280° C. The free base was prepared by treating the hydrogen iodide with alkali and extracting with chloroform, drying the extract with magnesium sulphate, and distilling off the solvent. The residual oil would not crystallize and was heated under reduced pressure in a cold-finger apparatus. The base was deposited on the condenser as a solid, m.p. 128° C ; $[\alpha]_{20^\circ}^{D}$, −219° in water. Ing (1931) recorded m.p. 134°. Power & Salway (1913) recorded $[\alpha]^{D}$, −221.6° in water, temperature not specified.

Caulophylline methiodide was prepared by heating the base under reflux in ethanol with an excess of methyl iodide. Recrystallized material had m.p. 265.5° C ; $[\alpha]_{20^\circ}^{D}$, −137.9° in water ; found ; I^-, 36.75 ; calculated for $C_{13}H_{19}ON_2I$; I^-, 36.65%. Ing (1931) recorded m.p. 276° (dec).

All the recrystallized material used for biological testing appeared to be homogeneous when chromatographed on paper in a solvent system consisting of butanol, ethanol and water (5:5:2) and developed with a modified Dragendorff reagent (Thies & Reuther, 1954). The materials also all showed an ultraviolet absorption maximum in water at 303 mμ, with log. $\varepsilon = 3.81$, and in the infrared, absorption maxima corresponding to the α-pyridone group occurred at 1650, 1555 and 1567 cm^{-1}.

The nicotine used was the (−)-isomer supplied as the hydrogen tartrate by British Drug Houses Ltd.

Dissociation constants. The pK_a values were determined by the method of Albert & Goldacre (1943). The procedure was the same as that of Barlow & Hamilton (1962) but with a stream of nitrogen in place of a stirrer driven by compressed air and with a Pye Dynacap instrument in place of the Marconi pH meter.

Biological

Toxicity. The acute toxicity of the compounds by intravenous, intraperitoneal and oral administration was studied in female albino mice, strain CS1, weighing between 17 and 24 g. These were divided into groups of ten and the animals in each group all received the same dose of the same drug by the same route, the dose being expressed as μmoles/kg, based on the average weight of the group. By each route each drug was tested using at least five different dose-levels ; in other words, at least five groups of mice were used for each drug given by one particular route. The mice were observed for 1 hr after dosing, and from the number which died in this period, the LD50 was calculated by the method of Litchfield & Wilcoxon (1949). The dose-levels were chosen so that, as far as possible, they were uniformly above and below the LD50 and, in all, about 50% of the animals died in the tests with one particular drug given by one particular route.

In these experiments nicotine was tested as the hydrogen tartrate, cytisine as the hydrochloride, and caulophylline as the hydrogen iodide, made up in 0.9% saline.

Ganglionic preparations. The superior cervical ganglion preparation of the cat, anaesthetized with chloralose, was set up as described by Paton & Perry (1953).

The preganglionic sympathetic nerve was stimulated with rectangular wave pulses of 0.7 msec duration at a rate of 10 shocks/sec. Usually a stimulus of 3–5 V was necessary to produce a maximum contracture of the nictitating membrane. Injections were made retrogradely into the external carotid artery, though in one instance the lingual artery was used. The blood pressure was recorded from a femoral artery. The relative blocking activity of the compounds was estimated by comparing the doses which produced roughly comparable block of the responses of the nictitating membrane to continuous stimulation of the preganglionic nerve. The relative stimulant activity of the compounds was estimated in a 2+1 assay by comparing the doses which produced comparable contractions of the nictitating membrane (in the absence of stimulation of the preganglionic nerve).

The relative ability of compounds to raise arterial blood pressure was studied in rats anaesthetized with urethane and cats anaesthetized with chloralose. The blood pressure was recorded from a carotid artery and the drugs were injected into a femoral vein, in a volume not exceeding 0.2 ml. and washed in with 0.9% saline.

The isolated guinea-pig ileum was set up in Tyrode solution at 37° C and bubbled with air. The volume of the organ bath was approximately 10 ml. and the drugs were added by pipette in a volume not exceeding 0.4 ml., allowed to act for 30 sec and then washed out. The interval between doses was 2 min.

Striated muscle. The chick biventer-cervicis preparation was set up, as described by Ginsborg & Warriner (1960), in Krebs–Henseleit solution (Krebs & Henseleit, 1932) at 37° C bubbled with 95% oxygen and 5% carbon dioxide. The nerve in the tendon was stimulated with rectangular wave shocks, which produced maximal twitches, of 0.7 msec duration at 6–8 shocks/min. The volume of the bath was approximately 30 ml. and the drugs were added by pipette in a volume not exceeding 0.4 ml., allowed to act until the contracture had reached a maximum (usually for about 10 min) and then washed out. Recovery usually occurred rapidly so the interval between doses was about 15 min. The compounds had a marked effect on the slow fibres, which masked any effects they may have had on the twitch responses. Their relative activities on this preparation were therefore estimated by comparing the doses which produced roughly the same degree of contracture.

The rectus abdominis muscle of the frog (*Rana pipiens*) was set up at room temperature in a modified Ringer solution bubbled with air. This solution had the following composition (in 1 l.): sodium chloride, 7.50 g (128 m-equiv); potassium chloride, 0.14 g (1.9 m-equiv); calcium chloride, 0.12 g (2.2 m-equiv); Tris (2-amino-2(hydroxymethyl)propane-1:3-diol), 1.92 g (15.9 m-equiv); to which was added 0.1 N hydrochloric acid; 14 ml. produced a pH of 7.10; 10 ml. produced a pH of 7.90; 9.4 ml. produced a pH of 8.20. The pH of the fluid in contact with the tissue differed slightly from these values and in every experiment samples were removed after contact for 4.5 min and their pH measured with a Pye Dynacap pH meter. Although there were differences of about 0.2 units between the pH of a particular buffer in different experiments, the variation did not exceed 0.02 units during any one experiment.

Equipotent molar ratios for the compounds relative to the quaternary salt, β-pyridylmethyltrimethylammonium, were obtained by 2+1 and 2+2 assay techniques using an automatic apparatus (Barlow, Scott & Stephenson, 1967). The drugs, made up in the desired concentration, were allowed to act on the preparation for 4.5 min and the interval between doses was 30 min. When an assay had been

completed, the pH was altered and a concentration of the quaternary compound was applied repeatedly until the responses were regular, indicating that the tissue had become adjusted to the new medium. This usually took less than an hour—that is, the second and third responses to the concentration of quaternary compound after the change were usually consistent. The assay was then repeated at the new pH.

The rat diaphragm preparation was set up exactly as described by Bülbring (1946), in Tyrode solution at 37° C bubbled with 95% oxygen and 5% carbon dioxide. The phrenic nerve was stimulated with rectangular wave shocks, which produced maximal twitches, of 0.75 msec duration at 5 shocks/min. The volume of the bath was approximately 25 ml. and the drugs were added by pipette in a volume not exceeding 0.4 ml., allowed to act until the block was fully developed and then washed out. The interval between doses was between 5 and 20 min. The relative activity was estimated in a 2+1 assay by comparing the doses which produced comparable degrees of block.

The anterior tibialis muscle preparation of the cat, anaesthetized with chloralose, was set up as described by Brown (1938). The peroneal nerve was stimulated with rectangular wave pulses, which produced maximal contractions of the muscle, of 0.7 msec duration at a rate of 6–8 shocks/min. The drugs were injected, in a volume not exceeding 0.1 ml., retrogradely into the anterior tibial artery. The blood pressure was recorded from a carotid artery. Measurement of blocking activity was usually by a 2+1 assay method, but in one experiment only approximate estimates were obtained by comparing the doses which produced roughly 50% block.

Respiration. Rabbits were anaesthetized with urethane and a cannula inserted into the trachea. This was connected to a respirometer (Gaddum, 1941) so that both the rate of respiration and changes in the volume of inspired air could be recorded. The blood pressure was recorded from a carotid artery. The drugs were injected in a volume not exceeding 0.2 ml. into a femoral vein and washed in with 0.9% saline. The interval between doses was 5 min and approximate estimates of relative activity were made by comparing the doses which produced roughly the same increase in the rate of respiration.

Acetylcholinesterase. Purified acetylcholinesterase, from ox red cells, was obtained from Nutritional Biochemicals Corporation. The effects of the compounds on the hydrolysis of acetylcholine (10^{-3}M) by this enzyme were studied by manometric methods as described by Barlow & Zoller (1964).

Results

Estimates of the LD50 of nicotine, cytisine and the methylated derivatives of cytisine, are shown in Table 1. Those mice which did not die within 1 hr were counted as survivors. Occasionally an animal treated with nicotine died many hours afterwards, but this never happened with the other drugs. The time after administration at which death occurred was noted and the average time of death for the animals killed in each group is indicated.

With nicotine the onset of symptoms was much faster than with the other compounds and the convulsions were qualitatively different. The tail became erect, rather like the Straub reaction, breathing appeared to be difficult, and there was frothing at the mouth, which was held wide open. The head was initially held

high, but subsequently forced down upon the chest. A few seconds before death the body became rigid, except for rapid trembling motions of the limbs.

With cytisine, caulophylline and caulophylline methiodide the convulsions were much slower in onset and much less severe, but there were more pronounced tonic and clonic contractions, particularly of the hind limbs. The body did not become as rigid as with nicotine and, just before death, the hind legs were stretched right back. All the animals which survived remained sedated for between 15 min and 1 hr afterwards.

The results of the tests on the other preparations are summarized in Table 2. Cytisine was invariably more active than caulophylline and usually more active than nicotine. Caulophylline methiodide was almost inactive and, in most tests, no effects were observed even with very large doses.

TABLE 1. *Acute toxicity*

		Route of administration		
		i.v.	i.p.	Oral
Nicotine	LD50 μmoles/kg	1·92	59·0	1,425
	limits	1·75–2·12	53·6–65·0	1,370–1,486
	LD50 mg/kg	0·3	9·5	230
		(6)	(7)	(9)
	Time of death	32·0±0·8 sec (28)	2·41±0·1 min (38)	2·86±0·1 min (45)
	e.p.m.r.	1	1	1
Cytisine	LD50 μmoles/kg	9·10	49·5	535
	limits	7·9–10·5	46·6–57·5	411–696
	LD50 mg/kg	1·73	9·4	101
		(6)	(6)	(7)
	Time of death	37·2±3·1 sec (36)	5·32±0·4 min (28)	12·7±0·6 min (46)
	e.p.m.r.	4·75	0·84	0·37
Caulophylline	LD50 μmoles/kg	103	252	>2,500
	limits	89·9–118	210–302	
	LD50 mg/kg	21	51	
		(6)	(5)	
	Time of death	41·3±1·4 sec (31)	6·92±0·4 min (26)	
	e.p.m.r.	53·6	4·27	
Caulophylline methiodide	LD50 μmoles/kg	280	>2,000	>8,000
	limits	239–328		
	LD50 mg/kg	61		
		(6)		
	Time of death	79·5±0·3 sec (32)		
	e.p.m.r.	146		

The table shows the LD50 in μmoles/kg and the fiducial limits ($P=0·05$). The value in mg/l g is shown below. The number of dose levels tested—that is, the number of groups of mice used—is shown in parentheses. The mean time after administration at which death occurred is shown±the standard error with the total number of animals which died shown in parentheses. The lowest entry shows the equipotent molar ratio (e.p.m.r.) for the compound relative to nicotine, calculated from the values of LD50.

The effects produced by cytisine and caulophylline were similar to each other but were not always exactly the same as those produced by nicotine. Consequently although a quantitative comparison of cytisine with caulophylline was possible, a quantitative comparison with nicotine is of doubtful value in some tests. The difference was most noticeable with the cat tibialis preparation, where the blocking action of nicotine lasted much longer than that of cytisine and caulophylline (Fig. 2). To a lesser extent this was true of the blocking action of the compounds on the cat superior cervical ganglion (Fig. 3). Caulophylline methiodide, in high doses, caused a block on this preparation but this was never accompanied by an initial stimulation. It is probably the result of a different type of action and so this compound cannot really be compared with the others. After the block there was an increase

TABLE 2. *Equipotent molar ratios for the compounds relative to (−)-nicotine in the various tests*

Preparation		Cytisine	Caulophylline	Caulophylline methiodide	Effective dose/animal or concentration of (−)-nicotine
Cat superior cervical ganglion	Stimulation	0·75±0·15 (6)	14·2±2·8 (6)	—	10 n-moles
	Block	1·17±0·07 (4)	20·1±2·2 (4)	27·0±3·9 (4)	100 n-moles
Blood pressure	Cat (rise)	0·54±0·11 (4)	5·60±0·81 (4)	—	300 n-moles
	Rat (rise)	0·41±0·14 (10)	0·84±0·24 (10)	—	50 n-moles
Guinea-pig ileum	(Contraction)	0·25±0·02 (3)	2·80±0·18 (3)	—	10^{-5}M
Chick biventer	(Contracture)	6·25±0·48 (4)	25·1±7·2 (4)	Very feeble (2)	10^{-6}M
Frog rectus	(Contracture)	0·64 (6)	2·7 (6)	—	10^{-5}M
Rat diaphragm	(Block)	0·66 (2)	15·4 (2)	Feeble	2×10^{-4}M
Cat tibialis	(Block)	*0·23±0·02 (3)	*2·40±0·19 (3)	—	300 n-moles
Rabbit respiration	(Increase)	3·05±0·33 (4)	27·7±2·8 (4)	—	1 μmole·

The mean is given±the standard error, with the number of experiments shown in parentheses. Figures for the cat tibialis, marked with an asterisk, are of doubtful value because of the difference in the time-course of the effects (see text), though they give a fair comparison of cytisine and caulophylline. Only a rough comparison was possible on the rat diaphragm preparation because the compounds were not very active and large amounts of material were needed. The figures for the frog rectus have been calculated from separate comparisons of the compounds with β-pyridylmethyltrimethylammonium and of this compound with (−)-nicotine. The final column shows the approximate dose or concentration which was effective in these experiments.

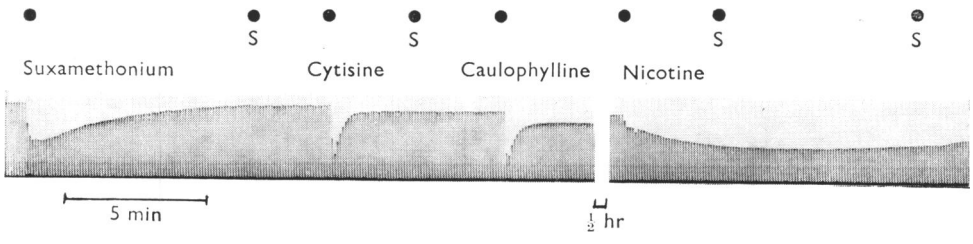

FIG. 2. Effects on the cat tibialis muscle. The record shows contractions of the tibialis muscle in response to stimulation of the peroneal nerve. Compare the transient blockade produced by cytisine (200 n-moles), caulophylline (2,000 n-moles) with the longer effects of suxamethonium (5 n-moles) and the prolonged effects of nicotine (400 n-moles). S indicates an injection of 0.9% sodium chloride (0.2 ml.).

in the size of the contracture ; this supramaximal response could well be the result of inhibition of cholinesterase by the high concentrations of caulophylline methiodide used (see later).

The results of the comparisons on the rat blood pressure were very variable but this variation can probably be ascribed to the preparation rather than to differences

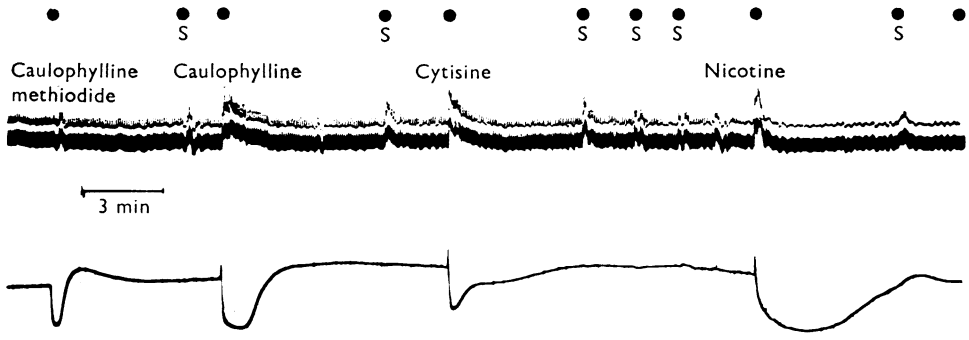

FIG. 3. Effects on the cat superior cervical ganglion. The upper record shows the blood pressure and the lower record the contracture of the nictitating membrane in response to stimulation of the preganglionic nerve. Cytisine (75 n-moles), caulophylline (1,000 n-moles), caulophylline methiodide (1,000 n-moles) and nicotine (75 n-moles) all produced comparable degrees of block. Note that cytisine, caulophylline and nicotine increased contracture before the onset of block and that the effects of nicotine lasted longer than those of cytisine and caulophylline. Caulophylline methiodide did not cause an increase in contracture before the onset of block, but did so afterwards. Note the effects of the compounds on the blood pressure. S indicates an injection of 0.9% sodium chloride (0.2 ml.).

TABLE 3. *Effects of pH on the activity of cytisine on the frog rectus*

	Equipotent molar ratio at			Proportion of	
	More acid pH	More alkaline pH	Ratio *a*/*b*	(*d*) ion present at more acid pH	(*e*) base present at more acid pH
pH	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)
7·88		8·35	1·39	1·53	0·25
7·10	6·00				
7·10	5·90				
7·88		7·20	1·22	1·53	0·25
7·88		9·45			
7·10	5·45		1·73	1·53	0·25
6·98	3·76				
7·88		7·65	2·04	1·57	0·20
7·92		7·65			
7·22	5·18		1·48	1·52	0·32
7·12	3·80				
7·9		5·00	1·31	1·54	0·26

Equipotent molar ratios for cytisine relative to β-pyridyl-methyltrimethylammonium are shown (*a*, *b*), together with the pH in the experiments at which they were obtained. The ratio (*c*) of these equipotent molar ratios can be compared with the effect of the changes in the pH on the proportion of ion (*d*) and base (*e*) present.
The average of the values in column *c* is 1·53, and in column *d* is 1·54.

between the actions of the drugs, because it did not occur when the compounds were compared on the cat blood pressure.

Cytisine and caulophylline were without effect on the hydrolysis of acetylcholine by acetylcholinesterase in concentrations as high as 4×10^{-3}M, but caulophylline methiodide was weakly active. Estimates of the pI50 were, 2.82, 2.82, 2.65 (mean 2.76 ; substrate concentration 10^{-3}M).

The pK_a values at 25° C were 7.92 for cytisine and 7.04 for caulophylline ; three estimates were made with each compound and gave identical results.

Effects of pH on activity on the frog rectus

Table 3 shows the equipotent molar ratios for cytisine relative to the quaternary compound, β-pyridylmethyltrimethylammonium, at different pH values. The increased activity in the more acid pH suggests that it is the cytisine ion which is the active species and, quantitatively, the effects on the equipotent molar ratio are in good agreement with the effects on ionisation, calculated from the pH and pK_a. On average the equipotent molar ratio at the more acid pH is 1/1.53 times that at the more alkaline pH, and the proportion ionized at the more acid pH is 1.54 times that at the more alkaline. In these experiments it did not seem to matter whether cytisine was tested in the more acid medium first or in the more alkaline.

Table 4 shows the results of similar experiments with caulophylline. Like cytisine this compound is clearly more active at the more acid pH, but the quantitative

TABLE 4. *Effects of pH an the activity of caulophylline on the frog rectus*

pH	Equipotent molar ratio at: More acid pH (*a*)	Equipotent molar ratio at: More alkaline pH (*b*)	Ratio *a*/*b* (*c*)	Proportion of: (*d*) ion present at more acid pH (*d*)	Proportion of: (*e*) base present at more acid pH (*e*)
7·43	33				
8·03		79·6	2·42	2·93	0·74
7·13	22·8				
8·20		130	4·84	6·15	0·53
7·13	25·9				
8·20		125	5·70	6·15	0·53
8·13		79			
7·15	45		1·75	5·20	0·53
8·13		81·3			
7·08	39·3		2·07	5·61	0·50
8·20		168			
6·87	49·7		3·38	7·16	0·43
8·14		100			
6·94	32·3		3·07	6·59	0·41

Equipotent molar ratios for caulophylline relative to β-pyridylmethyltrimethylammonium are shown (*a*, *b*) together with the pH in the experiments at which they were obtained. The ratio (*c*) of these equipotent molar ratios can be compared with the effect of the changes in the pH on the proportion of ion (*d*) and base (*e*) present. The experiments in which caulophylline was tested at the more acid pH first are shown in the upper section of the table.

agreement between the observed and calculated changes is much better in the experiments where the more acid pH was used before the more alkaline. In similar experiments with nicotine tested at different pH values on the frog rectus, Hamilton (1963) observed exactly the same phenomenon; the quantitative agreement was much better when the more acid pH was used first.

Discussion

The results of the toxicity experiments indicate that cytisine is less toxic than nicotine intravenously but more toxic by intraperitoneal or oral administration. By any of these routes, however, it does not appear to be particularly lethal in mice. The intravenous LD50 of cytisine, 1.73 mg/kg, corresponds to 120 mg/70 kg and the equivalent of the oral LD50 would be 7 g/70 kg. In two experiments, doses of cytisine 1.2 times the LD50 in mice were tested on guinea-pigs and rats and did not cause death. The animals appeared to be sedated, but not distressed, so there seems to be no reason to believe that mice are particularly resistant to cytisine compared with other rodents.

The likely toxic dose in man, of course, is difficult to predict, especially as cytisine can cause vomiting. The doses used in many of the pharmacological tests are low, so quite small amounts might produce effects which were unpleasant, but the experiments with animals suggest that these are not likely to be lethal. Instances of death from laburnum poisoning were reported in the last century (Radziwillowicz, 1888) but although there have been many cases of poisoning this century, none of these seems to have been fatal (Mitchell, 1951). In forty-four enquiries about laburnum poisoning to the Scottish Poisons Information Bureau between May 1963 and May 1968 there was complete and uneventful recovery in all instances.

The high LD50 values for nicotine by the oral route were surprising in view, for example, of Gaddum's statement (1953) that " if a couple of drops of pure nicotine are placed on a dog's tongue the dog drops down dead in a few seconds." Estimates of the LD50 for nicotine, however, vary very considerably (Larson, Haag & Silvette, 1961) and it seems that, by the oral route, solutions of salts of nicotine are less toxic than solutions of the base. We may have obtained high values because we used a salt and also because we placed the dose directly into the stomach. The acid environment will greatly delay absorption and the use of a stomach-tube to administer the drug will prevent any absorption through the mucous membranes of the buccal cavity, which might otherwise occur during its passage from the mouth to the stomach. Absorption in this way, between the mouth and the stomach, might well account for the discrepancy between values of the oral LD50 for nicotine (see above). At normal body pH, there is rapid absorption of nicotine base across membranes and, if the dose is not placed directly in the stomach, a considerable proportion may be absorbed before it reaches an acid environment. There should be much less absorption of nicotine from solutions of the salts, especially if these are acid (such as the hydrogen tartrate). The toxicity of cytisine may be similarly dependent on whether it is tested as the base or as the salt.

From the results of the experiments in mice it seems that death from cytisine either occurs rapidly or not at all (see Table 1) which suggests that the body is able to tolerate or detoxicate the drug if it is slowly absorbed. Larson, Finnegan & Haag (1949) have, in fact, shown that when nicotine is given intravenously by infusion over a period of 8 hr, some animals are able to tolerate up to 11 times the

amount which would be lethal in a single injection. In view of this it could be argued that it is unnecessary to wash out the stomach of children who have eaten laburnum, or other plants containing cytisine, especially as the alkaloid itself is likely to cause vomiting. It might be much more important to wash out the mouth with an acidic buffer.

Our results with the various tests confirm the resemblance between the pharmacological properties of cytisine and nicotine observed by Dale & Laidlaw (1912). They also confirm the conclusions of Zachowski (1938), that cytisine is more powerful as a ganglion stimulant than as a ganglion blocking agent. The resemblance between the peripheral effects of cytisine and nicotine is striking and our results show that this resemblance is quantitative as well as qualitative. On the cat and rat blood pressure, the guinea-pig ileum, the frog rectus and the rat diaphragm, comparable effects are produced by doses of cytisine from one-quarter to two-thirds of those of nicotine. The results with the cat tibialis are not really comparable, because the effects of cytisine are only transient. The results with the chick biventer are noticeably different but it is possible that these indicate marked sensitivity of chick muscle to nicotine, rather than an insensitivity to cytisine.

From the differences between the types of convulsion produced by the two alkaloids, as well as from the absence of ear-twitches after cytisine noted by Dale & Laidlaw (1912), it seems that the similarity of the effects may not be so great in the central nervous system. The action of cytisine on the respiration of anaesthetized rabbits is certainly weaker than would be expected from its effects on the peripheral nervous system.

From the experiments at different pH with the frog rectus it appears that cytisine also resembles nicotine in being active as the ion. Equipotent ionic ratios should therefore be compared, rather than equipotent molar ratios. These are shown in Table 5, in which the mean values of the equipotent molar ratio from Tables 1 and 2 have been corrected for the degree of ionization of nicotine and of the compound. With cytisine this makes little difference, because the pK_a, 7.92, is only slightly less than that of nicotine, 8.01 (Barlow & Hamilton, 1962). Caulophylline, however, is a much weaker base, pK_a 7.04, and it is clear that part of the decline in pharmacological activity produced by methylating cytisine is due to the effect of methylation on basicity. The decline in basic strength is likely to be caused by steric hindrance ;

TABLE 5. *Equipotent ionic ratios*

Preparation		Cytisine	Caulophylline	
		(*a*)	(*a*)	(*b*)
Cat superior cervical ganglion	Stimulation	0·72	4·36	6·05
	Block	1·13	6·27	5·55
Blood pressure	Cat (rise)	0·53	1·72	3·25
	Rat (rise)	0·40	0·26	0·65
Guinea-pig ileum	(Contraction)	0·24	0·70	2·92
Chick biventer	(Contracture)	5·90	5·63	0·96
Frog rectus	(Contracture)	0·64	2·67	4·17
Rat diaphragm	(Block)	0·63	3·85	6·12
Cat tibialis	(Block)	0·22	0·74	3·36
Rabbit respiration	(Increase)	2·94	8·52	2·90

The equipotent molar ratios in Tables 1 and 2 have been corrected for the degree of ionization of the compounds. Figures in columns *a* are relative to the univalent nicotinium ion; those in *b* are for the caulophylline ion relative to the cytisine ion.

the methyl group, which is electron-releasing and therefore usually base-strengthening (see, for example, Clark & Perrin, 1964), restricts the access of hydrogen ions to the nitrogen atom and this is already limited by the bulk of the bridged-ring system (Fig. 1).

Even allowing for the difference in basicity, however, it is clear that the caulophylline ion is weaker than the cytisine ion. In most tests it has between one-half and one-sixth of the activity, though on the chick biventer it has the same activity as the cytisine ion and on the rat blood pressure it is even more active. Apart from these quantitative differences, however, the pharmacological effects of cytisine and caulophylline are indistinguishable.

The feeble activity of caulophylline methiodide is surprising. Apart from its weak effects as an inhibitor of acetylcholinesterase and as a ganglion-blocking agent, it seems to be inactive. It is toxic to mice only when very large doses are given intravenously, but the symptoms produced are similar to those of cytisine and caulophylline. We thought it possible that the introduction of the second methyl group into caulophylline might have altered the conformation of ring C, making the boat form preferable to the chair form shown in Fig. 1. Normally the boat form is thermodynamically less favoured, but it seemed that the change might occur

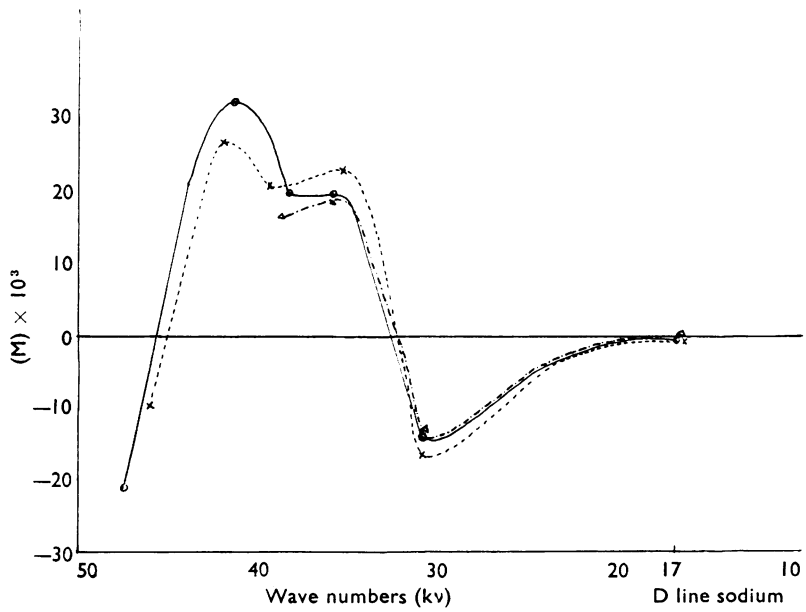

FIG. 4. Optical rotatory dispersion curves. Wave numbers ($k\nu$; 1 $k\nu$=1,000 cm^{-1}) are plotted against the molar rotations. Measurements were made with 2×10^{-4}M concentrations in distilled water. Similar results were obtained with the hydrogen iodides as with the bases except that above 40,000 wave numbers (250 mμ) the iodide absorption was so strong that the rotation could not be measured. The Cotton effect between 31,000 and 35,000 wave numbers corresponds to the peak absorption due to the pyridone chromophore, and is the same shape for all three compounds. The measurements were made with a Bellingham and Stanley Polarmatic 62 instrument, which scans continuously, but does not measure rotation directly. The absolute values were calculated for the points shown (×---×, caulophylline; ○——○, cytisine; △-·-·-△ caulophylline methiodide) and the intervening parts of the curves were drawn from the traces of the relative rotations recorded by the instrument.

because the basic nitrogen atom in ring C and the pyridine nitrogen atom in rings A and B are fairly close together. The introduction of a second methyl group, which must be placed between these two atoms, might tend to force them further apart but, to offset this there would then be considerable steric interference between the other methyl group on the nitrogen atom in ring C and an axial hydrogen atom. These changes in conformation involve the movement of groups close to the chromophore, the pyridone system (λ max, 303 mμ), and consequently might be expected to produce changes in the optical rotatory dispersion curves. These were examined, however, and found all to have the same shape (Fig. 4), and consequently the inactivity of caulophylline methiodide, either as an agonist or an antagonist, does not seem to be caused by any fundamental change in preferred conformation.

It seems, then, that the progressive methylation of cytisine decreases activity because it decreases the ability of the onium group, the nitrogen atom in ring C, to fit the receptor. This is likely to be a steric effect, due simply to the increase in size. Chemical support for this comes from the decreased basicity of caulophylline compared with cytisine, and also from the difficulty of preparing caulophylline methiodide, which is only obtained after heating caulophylline with methyl iodide for 48 hr. It is, however, possible that cytisine produces effects when only a small proportion of receptors is occupied and consequently has only a low affinity. The decline in activity with methylation would then indicate a decline in efficacy rather than in affinity. The absence of agonist activity in caulophylline methiodide clearly indicates a decline in efficacy, but the absence also of appreciable antagonist activity suggests that methylation has also decreased affinity unless the affinity of cytisine is very low indeed—that is, unless cytisine has a particularly high efficacy.

From molecular orbital calculations, Kier (1968) has deduced the preferred conformations of nicotine and acetylcholine and has suggested that the presence in a molecule of a quaternary nitrogen atom and a negatively charged atom situated 4.85 ± 0.1 Å away are key features for nicotine-like activity. In models the nitrogen atom in ring C of cytisine appears to be 4.8–9 Å away from the oxygen atom of the pyridone group, which will be partially negatively charged. This agreement, however, may be fortuitous. It is not clear whether the key features confer affinity or efficacy on a molecule or, more probably, a particularly desirable combination of the two properties. They cannot be the only criteria for activity, however, because though they are present in cytisine and caulophylline, they are also present in caulophylline methiodide, which is virtually inactive.

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