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# THE ADRENERGIC-NEURONE BLOCKING ACTION OF SOME COUMARAN COMPOUNDS

BY

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Ethyldimethyl(7-methylcoumaran-3-yl)ammonium iodide (SK&F 90,109) and its guanidine analogue [*N*-(7-methylcoumaran-3-yl)guanidine nitrate] (SK&F 90,238) abolish the effects of adrenergic nerve stimulation in cats, as do xylocholine and bretylium. SK&F 90,109 has slight sympathomimetic actions ; these are less marked than in SK&F 90,238. Large doses of SK&F 90,109 have an action, dependent on local noradrenaline stores, that delays the appearance of adrenergic-neurone blockade in conscious cats. Responses to adrenaline are, in general, enhanced by each drug, but SK&F 90,238 transiently antagonizes tachycardia induced by adrenaline and isoprenaline. Both drugs inhibit the release of noradrenaline from the spleen during splenic nerve stimulation, but the release of catechol amines from the adrenal glands, in response to electrical or chemical stimulation, is unimpaired. In contrast to the prolonged adrenergic-neurone blocking action, any inhibition of the effects of cholinergic nerve stimulation is transient. Large intravenous doses produce neuromuscular blockade. The compounds have a slight central depressant action. In contrast to reserpine and guanethidine the noradrenaline content of rat hearts is not appreciably lowered 24 hr after a single dose of either drug. Unlike xylocholine they are not local anaesthetics. Related compounds also block the effects of adrenergic-nerve stimulation. The possible modes of action of these drugs are discussed.

This paper describes the actions of some coumaran (2,3-dihydrobenzofuran) derivatives on the peripheral autonomic nervous system. The preparation and properties of the compounds studied, which are derived from generic structure I, will be described later in the Chemical Appendix. It will be apparent from Fig. 1 that the structures of the two compounds whose actions are to be described in detail, namely SK&F 90,109 [ethyldimethyl(7-methylcoumaran-3-yl)ammonium iodide] and SK&F 90,238 [*N*-(7-methylcoumaran-3-yl)guanidine nitrate] are related to that of xylocholine (TM10). All the compounds prepared have an asymmetric carbon atom, and are thus capable of resolution, but they have all been studied as racemic mixtures.

## METHODS

### *Experiments on anaesthetized cats*

Experiments were done on the nictitating membranes, heart, pilomotor muscles, uterus, spleen, stomach, blood vessels, salivary glands, sweat glands and adrenal glands of anaesthetized cats. Anaesthesia was induced with ether, or with ethyl chloride followed by ether, and maintained with chloralose (80-100 mg/kg intravenously). Drugs were given through a metal cannula tied into a femoral vein. Blood pressure was recorded from a suitable artery with a mercury manometer.

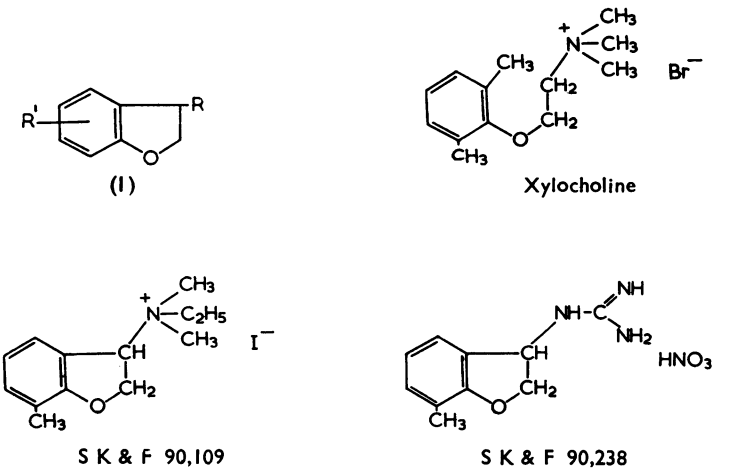



Fig. 1. The generic structure (I) of the compounds studied, together with that of xylocholine and the two compounds examined in detail.

*Electrical stimulation.* Stimulation of nerve trunks was by rectangular pulses of 0.5 msec duration through shielded, bipolar platinum electrodes. In most experiments the frequency was 50 shocks/sec and the voltage was supramaximal. An isolation transformer was interposed between the stimulator and the electrodes.

*Nictitating membranes.* The movements of both nictitating membranes were recorded by frontal writing levers. The postganglionic trunk of the right and the preganglionic trunk of the left cervical sympathetic nerve were prepared for stimulation. The postganglionic nerve was approached by removal, between ligatures, of the larynx and sections of the trachea and oesophagus. Periodic stimulation of the nerves and injection of adrenaline were effected automatically. Microswitches operated by cams driven by a synchronous electric motor switched the output of the stimulator to each electrode in turn for periods of 10 sec every 2 min for 10 min. Then followed a period of 8 min without stimulation. Halfway through this latter period a Palmer slow-injection apparatus was switched on for a few seconds, injecting, through the femoral cannula, a solution of adrenaline.

*Heart rate.* The thorax was opened by removing the sternum. After cutting the pericardium the heart was attached to a Cushney myocardiograph. This operated either a lever writing on a fast moving drum, or a switch which operated a Thorp impulse counter. The right stellate ganglion was exposed, the preganglionic nerve was cut, and the inferior cardiac nerve was laid across electrodes. The left vagus nerve was cut and the distal end was placed on electrodes.

*Uterine movements.* After removing the intestines from a nonpregnant cat the cervical end of the right horn of the uterus was fixed *in situ* and the other end was freed from the ovary and connected by thread to a heavily loaded lever. Both hypogastric nerves were prepared for stimulation. The opened abdomen was covered with moist swabs, heated by an infrared lamp.

*Splenic venous blood.* This was collected by the method of Brown & Gillespie (1957). The greater splanchnic nerves were cut, the splenic nerves were separated, tied proximally, and placed across electrodes. A loose nylon ligature was placed round the portal vein between the junction of the splenic and superior mesenteric veins and the entry of the gastric vein. The uperior mesenteric artery was cannulated with polyethylene tubing. Heparin (500 U/kg) was

given by vein. Blood was diverted from the spleen into the cannula by pulling on the nylon ligature. Blood was collected in cooled, silicone-coated, graduated tubes containing 200 U of heparin. The splenic nerves were stimulated for 15 sec and blood was collected for the period of stimulation plus the first 5 sec of the post-stimulation period. Resting samples were collected for 20 sec. Samples were immediately spun, at 5° C for 15 min at 3,000 *g*, the volumes of plasma and cells were noted and the plasma was removed. The plasma was kept at 5° C and assayed as soon as possible for pressor activity in pithed rats, using (−)-noradrenaline bitartrate as standard.

Rats (150 to 200 g) were given 1 mg of atropine sulphate subcutaneously, anaesthetized with pentobarbitone sodium (30 mg/kg, intraperitoneally) and pithed by passing a steel wire through the right orbit and down the vertebral canal. The animals were ventilated by a small pump which supplied air at a constant pressure through electromagnetic valves controlled by a transistor asymmetrical-multivibrator so that the period of inflation was one-third that of deflation. The rate of ventilation and the pressure of the air supplied were variable, but typical values were 120 inflations/min and 20 cm of water respectively.

*Stomach movements.* A polyethylene tube was passed into the stomach through an incision in the oesophagus and tied in position. The duodenum was ligated. After washing out the stomach it was filled with 0.9% saline at 37° C, and the tube was connected to a sensitive diaphragm-type pressure transducer, the output of which was displayed on a potentiometric chart recorder (Sunvic 10 S). The sympathetic nerves were separated from the gastric artery and laid across electrodes; the ventral oesophageal branch of the vagus nerve was also prepared for stimulation.

*Blood flow.* The left lumbar sympathetic nerve trunk was tied below the 4th lumbar ganglion and the distal portion was placed on electrodes. Heparin (1,000 U/kg) was given by vein. Central and distal cannulae were placed in the left femoral artery, and a modified version of the density flowmeter (Dawes, Mott & Vane, 1953) was placed in the circuit. The right femoral artery was cannulated and connected to a reservoir of blood, collected from another cat, diluted with one-half to one-third its volume of 0.9% saline. This reservoir was placed so that its hydrostatic pressure was about equal to the cat's blood pressure. The left leg was placed in a Perspex plethysmograph fitted with a moulded latex cuff. The plethysmograph was filled with water at 37° C and connected to a volume recorder. The temperature was kept constant with an infrared lamp.

In experiments on splanchnic blood flow the flowmeter was placed in the superior mesenteric artery and the periarterial nerves were prepared for stimulation.

*Salivary secretion.* The right submaxillary duct was cannulated and the saliva was allowed to displace saline, containing a little detergent, from a reservoir through a fine-bore drop-tube. Each drop was recorded by an electromagnetic signal marker driven by a transistor-amplifier. The chorda tympani and cervical sympathetic nerves were prepared for stimulation.

*Nerve action potentials.* These were picked up by platinum electrodes and passed into an AC-coupled amplifier. The output was displayed on a Tektronix 502 cathode-ray oscilloscope and photographed by a Grass C4 recording camera.

*Experiments on conscious cats.*

Cats were given drugs by mouth (in gelatin capsules), by vein or, more usually, by subcutaneous injection. Photographs were taken at intervals to record any relaxation of the nictitating membranes.

*Finkleman preparations*

Lengths of rabbit ileum with attached mesentery and sympathetic nerves (Finkleman, 1930) were suspended in Tyrode solution at 37° C. Pendular movements were recorded. The mesentry was stimulated for 30 sec every 10 min. The periodic stimulation, control of the kymograph, and the changes, by displacement, of bath fluid were done automatically.

RESULTS

*Effects of SK&F 90,109 and 90,238 on the nictitating membranes.* Intravenous injection of 10 mg/kg of SK&F 90,109 greatly reduced or abolished the contractions of the nictitating membranes evoked by stimulation of either the pre- or the postganglionic trunks of the cervical sympathetic nerves. The development of the block was gradual, often taking 60 to 120 min to become complete. When established the block persisted for several hours. Cocaine hydrochloride (2.0 mg/kg) caused some reversal of the block. Responses to adrenaline were increased. SK&F 90,238 had similar actions. This compound also caused a marked and long-lasting contraction of the nictitating membranes, usually associated with a prolonged rise in blood pressure. Large doses of SK&F 90,109 (100 mg/kg) also produced marked and prolonged contractions of the nictitating membranes. Fig. 2 shows some of these actions of SK&F 90,109 and 90,238. These compounds, therefore, abolished for long periods the effects of sympathetic nerve stimulation without antagonizing the effects of adrenaline.

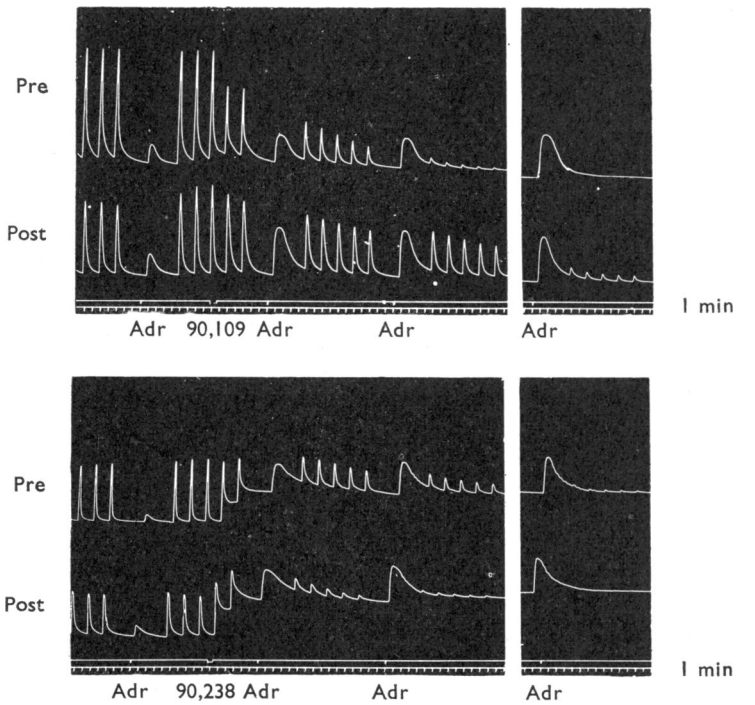



Fig. 2. Effect of 10 mg/kg of SK&F 90,109 (upper records) and SK&F 90,238 (lower records) on the contractions of the nictitating membranes of cats (2.5 and 2.3 kg respectively) treated with atropine and anaesthetized with chloralose. Adrenaline (10 $\mu$g) was injected intravenously at Adr. The preganglionic (upper traces) and postganglionic (lower traces) cervical sympathetic nerves were stimulated alternately for 10 sec every 2 min except when adrenaline was injected. The records on the right were started 2 hr after the injection of SK&F 90,109 and of 90,238. Responses to pre- and postganglionic stimulation were gradually abolished and the effect of adrenaline was increased.

Given to conscious cats, both drugs caused a relaxation of the nictitating membranes and a narrowing of the palpebral fissure, but the size of the pupil did not change. After subcutaneous injection, SK&F 90,109 and 90,238 were approximately equiactive, so that after a dose of 10 mg/kg about one-third of the eye was covered.

Both drugs were effective when administered either by vein or by mouth. By the latter route absorption seemed good, so that only two to three times the effective subcutaneous dose was required to produce a similar response.

With subcutaneous doses up to 20 mg/kg SK&F 90,109 produced a maximal effect some 3 to 8 hr after injection, whereas it took 12 to 24 hr for SK&F 90,238 to give full relaxation of the nictitating membranes. Both drugs acted for a long time, the effects of 20 mg/kg, subcutaneously, persisting for 2 to 3 days. However, when larger doses of SK&F 90,109 were given to cats the time until the nictitating membranes were maximally relaxed progressively increased as the dose of drug was increased. The results of one such experiment are illustrated in Fig. 3. The

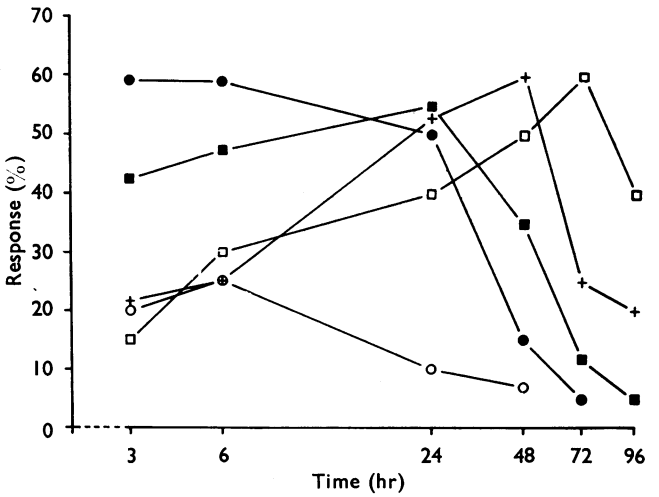



Fig. 3. Time/response curves of SK&F 90,109 following subcutaneous injection into five conscious cats. The response is expressed as the percentage of the lower eye margin covered by the relaxed nictitating membrane. For explanation see text. ○——○, 10 mg/kg; ●——●, 20 mg/kg; ■——■, 40 mg/kg; +——+, 80 mg/kg; and □——□, 160 mg/kg.

injection of 10 or 20 mg/kg caused maximum relaxation some 6 hr after injection, whereas it was 3 days before the full effects of 160 mg/kg were apparent. Intermediate doses (40 and 80 mg/kg) produced their full effects between 1 and 2 days after administration. But in anaesthetized cats the subcutaneous injection of 100 mg/kg of SK&F 90,109 abolished, within 30 min, the contractions of the nictitating membranes evoked by electrical stimulation of the cervical sympathetic nerves, whether stimulation was continuous at a frequency of 2 shocks/sec, or periodic (15 sec every 2 min) at 50 shocks/sec.

SK&F 90,109 was given to several cats in which one nictitating membrane had been relaxed by cutting, 7 days earlier, the preganglionic cervical sympathetic nerve

(" decentralization "). There was no effect on the " decentralized " nictitating membrane after 20 mg/kg, subcutaneously, of SK&F 90,109. Larger doses (40 and 80 mg/kg) completely retracted, within 60 and 30 min respectively, the relaxed nictitating membranes. This effect persisted for several hours so that some 3 hr after the injection of the higher doses the " decentralized " membranes were fully retracted and the innervated membranes partially relaxed. At 24 hr after administration, both normal and " decentralized " membranes were well relaxed. SK&F 90,109 had, therefore, in conscious cats, two opposing actions on the nictitating membranes, the one that caused the contraction of the muscles persisting for a shorter time than the adrenergic-neurone blocking action.

The retraction of the " decentralized " nictitating membranes after SK&F 90,109 (100 mg/kg, subcutaneously) was not prevented by treating the cats 1 to 3 hr beforehand with hexamethonium bromide (20 mg/kg, subcutaneously), with atropine sulphate (5 mg/kg, subcutaneously) or with phenoxybenzamine (2 or 5 mg/kg, by slow intravenous infusion), although after treatment with phenoxybenzamine intravenous injections of 10 $\mu$g of adrenaline still contracted the nictitating membranes. SK&F 90,109 did not, however, affect the normal or " decentralized " nictitating membranes of cats treated 24 hr earlier with reserpine (1 mg/kg, intraperitoneally). SK&F 90,109 was also given to a cat in which one nictitating membrane had been relaxed by cutting the preganglionic cervical sympathetic nerve (" decentralization "), and the other by removing the superior cervical ganglion (" denervation "). Five days after this operation SK&F 90,109 produced, 5 min after the injection, a small and transient contraction of the " denervated " nictitating membrane. After 30 min the " decentralized " membrane was fully withdrawn but the " denervated " membrane remained well relaxed. However, in an experiment on another cat in which the drug was given only 20 hr after removing the ganglion the nictitating membrane readily contracted, but when the treatment with drug was repeated 4 days later the response was much reduced.

*Effect of SK&F 90,109 and 90,238 on sympathetic inhibitory responses.* An experiment on the uterus of a nonpregnant cat is recorded in Fig. 4. The relaxations of the uterus caused by hypogastric nerve stimulation and by injecting 10 $\mu$g of adrenaline are shown. Some 40 min after an injection of 20 mg/kg of SK&F 90,109, which caused a marked contraction of the uterus, the response to nerve stimulation was greatly reduced but the inhibition caused by adrenaline was not affected. After a second dose of 20 mg/kg of SK&F 90,109 there was no response to nerve stimulation although adrenaline still relaxed the uterus. Uterine tone was slightly reduced after an injection of SK&F 90,238 (20 mg/kg) and the response to sympathetic nerve stimulation was slowly abolished. The effect of adrenaline was increased. These drugs, therefore, abolished the inhibitory responses of the uterus to sympathetic nerve stimulation but not to adrenaline.

The inhibition of the pendular movements of isolated lengths of rabbit ileum in response to periarterial nerve stimulation was abolished by adding SK&F 90,109 (10 $\mu$g/ml.) or SK&F 90,238 (5 $\mu$g/ml.) to the bathing fluid. The block survived many changes of bathing fluid. The responses to adrenaline were unaffected.

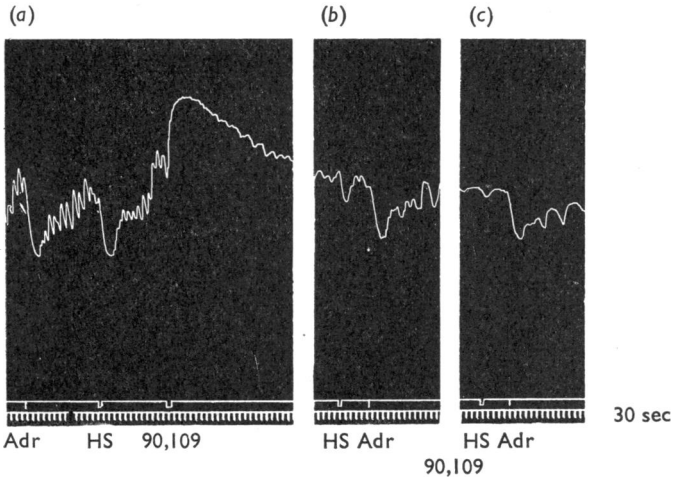



Fig. 4. Cat, nonpregnant female, 1.8 kg, anaesthetized with chloralose. Record of uterine tone At Adr, 10 $\mu$g of adrenaline injected intravenously, at HS stimulation of the hypogastric nerves for 15 sec (50 shocks/sec). Record (*b*) was started 40 min after injecting SK&F 90,109 (20 mg/kg) and record (*c*) was taken 40 min later and 20 min after a second injection of SK&F 90,109 (20 mg/kg). Responses to nerve stimulation, but not to adrenaline, were inhibited by SK&F 90,109.

*Effect of SK&F 90,109 and 90,238 on sympathetic and parasympathetic nerves.* SK&F 90,109 and 90,238 (10 mg/kg) readily blocked for long periods the effects of stimulating the sympathetic nerves to the heart. Immediately after injecting either of these drugs the slowing of the heart during vagal stimulation was greatly reduced, but only for a short time. Thus the effects of sympathetic nerve stimulation were inhibited when the activity of the parasympathetic nerves was unimpaired. The responses to adrenaline were not affected by this dose of SK&F 90,109, but were reduced, as were the responses to isoprenaline, immediately after the injection of SK&F 90,238. After 30 min adrenaline and isoprenaline produced their usual effects. The effects of sympathetic nerve stimulation were, therefore, blocked by these drugs at times when responses to sympathomimetic amines were unimpaired.

The results of two experiments on salivary secretion are illustrated in Fig. 5. The top records show the responses before giving drugs, to stimulating the cervical sympathetic nerve, the chorda tympani, and to intravenous adrenaline. At 15 min and 1 hr after injecting 10 mg/kg of SK&F 90,109 and of 90,238, respectively, the responses to sympathetic nerve stimulation were abolished, the responses to parasympathetic nerve stimulation were little changed but adrenaline evoked, particularly in the cat given SK&F 90,109, a much greater flow of saliva than before. Again, therefore, SK&F 90,109 and 90,238 selectively blocked the effects of sympathetic nerve stimulation. In other experiments the flow of saliva in response to stimulation of the chorda tympani was partly reduced for a short time.

The pressure in the stomach fell when the sympathetic nerves were stimulated for 15 sec (50 shocks/sec) and rose during stimulation of the parasympathetic nerves (50 shocks/sec). Some 15 to 30 min after giving 10 mg/kg of SK&F 90,109 or

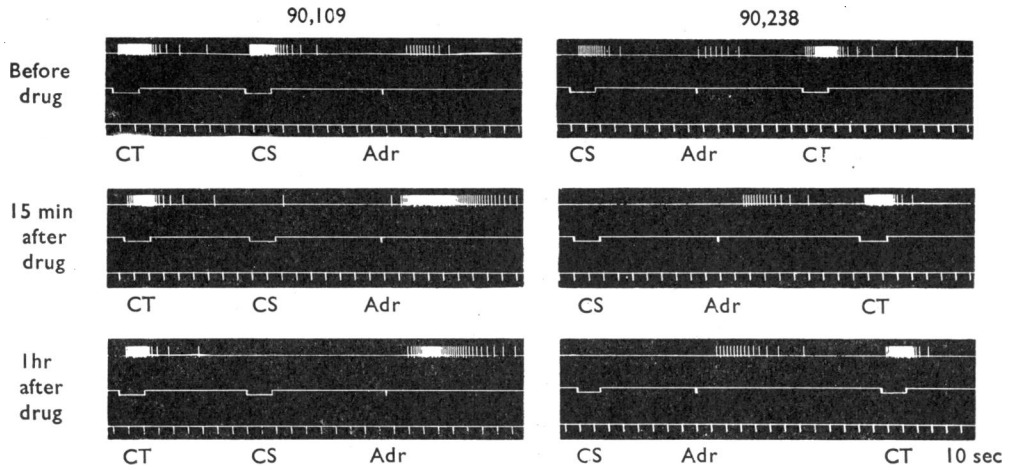



Fig. 5. Effects of SK&F 90,109 and of SK&F 90,238 on the salivary secretion from the submaxillary glands of cats in response to stimulating for 15 sec (50 shocks/sec) the chorda tympani nerves (CT), the cervical sympathetic nerves (CS), and to injecting 20 $\mu$g of adrenaline (Adr). The top records show the responses before drug treatment. The middle and bottom records were taken 15 min and 1 hr, respectively, after the administration of 10 mg/kg of SK&F 90,109 or SK&F 90,238. Each signal mark represents one drop of saliva. Each drug abolished the effect of sympathetic nerve stimulation, had little effect on the response to parasympathetic stimulation and enhanced the response to adrenaline.

90,238 the response to sympathetic nerve stimulation was abolished for the duration of the experiment. The effects of vagal stimulation were unaffected by SK&F 90,109 and but briefly reduced by SK&F 90,238.

The pilomotor muscles of the tail and the sweat glands in the hind-feet are innervated by the lumbar sympathetic nervous system. But, whereas the nerves to the muscles are adrenergic, those to the sweat glands are cholinergic (Dale & Feldberg, 1934). Stimulating the left lumbar sympathetic nerve trunk below the 4th lumbar ganglion for 30 sec caused erection of the hairs on the tail and the appearance of beads of sweat on the pads of the foot. Stimulating 15 and 60 min after injecting SK&F 90,109 or 90,238 (10 mg/kg) produced no piloerection, although sweating occurred as usual. These drugs therefore affected adrenergic but not cholinergic nerves.

*Effects of SK&F 90,109 and 90,238 on the cardiovascular system.* Intravenous injection of SK&F 90,109 and 90,238 caused variable effects on the blood pressure of supine anaesthetized cats. The usual response was a rise of from 30 to 60 mm Hg lasting for 15 to 30 min, after which the blood pressure returned to control levels, or sometimes to a little below. In some experiments, however, SK&F 90,238 caused only a fall in blood pressure of 30 to 40 mm Hg.

The fall in blood pressure of anaesthetized cats tilted from the horizontal to the vertical position was markedly increased after SK&F 90,109 (15 mg/kg) or 90,238 (20 mg/kg).

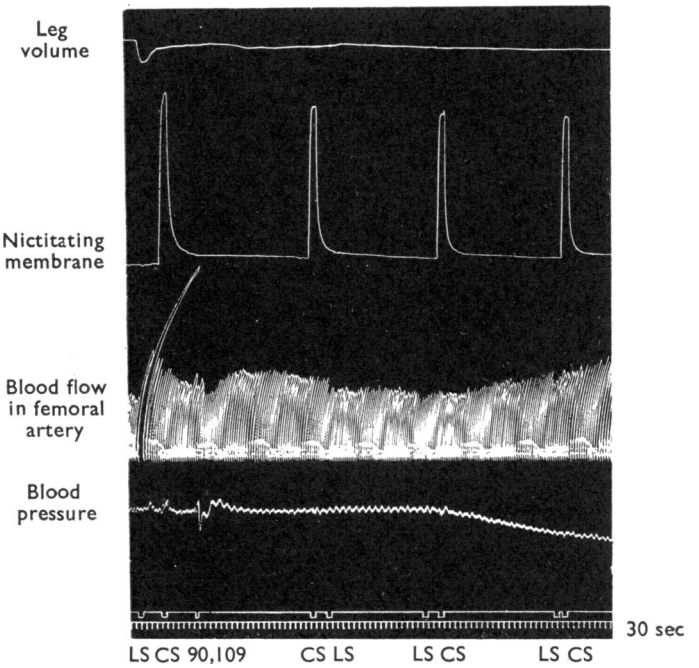



Fig. 6. Cat, 3.0 kg, anaesthetized with chloralose, showing contractions of the right nictitating membrane in response to stimulating for 15 sec (50 shocks/sec) the cervical sympathetic nerve (CS), and the changes in volume of the left hind-limb and blood flow in the left femoral artery during stimulation of the left lumbar sympathetic nerve (LS). The blood pressure was stabilized. SK&F 90,109 (5 mg/kg) abolished the effects of lumbar sympathetic nerve stimulation but had little effect on the contractions of the nictitating membrane.

Fig. 6 shows the results of an experiment on the blood vessels of the hind-limb. Stimulation of the lumbar sympathetic chain greatly reduced the flow in the femoral artery and reduced the volume of the leg. Stimulating the cervical sympathetic nerve contracted the nictitating membrane. About 10 min after 5 mg/kg of SK&F 90,109 the response of the nictitating membrane was hardly reduced but on stimulating the lumbar sympathetic nerves slight vasodilation occurred. Similarly, SK&F 90,238 readily abolished the vasoconstriction caused by sympathetic nerve stimulation to the hind-limbs.

The rise in blood pressure evoked by stimulating the nerves accompanying the superior mesenteric artery was greatly reduced or abolished after injecting SK&F 90,109 or 90,238. The amount of drug required to abolish this effect varied. A dose of between 10 and 20 mg/kg was usually sufficient, but in one experiment 40 mg/kg of SK&F 90,109 was needed. In experiments in which the vasomotor nerves to the intestine and kidney were intact, SK&F 90,238 (20 mg/kg) or 90,109 (20 mg/kg) greatly reduced, for long periods, the rise in blood pressure caused by stimulating the left splanchnic nerves. SK&F 90,109 readily abolished the reduction in blood flow in the superior mesenteric artery during periarterial nerve stimulation.

These drugs, therefore, readily blocked the effects of sympathetic nerve stimulation on blood vessels.

Changes in cardiac output were recorded by cannulating the pulmonary artery and recording blood flow with a density flowmeter (Barer & Nüsser, 1958). SK&F 90,109 (5 or 10 mg/kg) had no effect on cardiac output.

*Effects of SK&F 90,109 and 90,238 on the output of noradrenaline from the spleen.* Peart (1949) first showed that on stimulating the splenic nerves the noradrenaline content of splenic venous blood was increased. Xylocholine and bretylium abolish this effect (Exley, 1957 ; Boura & Green, 1959).

The results of experiments with SK&F 90,109 and 90,238 are shown in Fig. 7. Splenic nerve stimulation increased two- to threefold the noradrenaline content of blood collected from the spleen. However, samples collected 65 and 140 min after SK&F 90,109 (15 mg/kg) or 35 and 95 min after SK&F 90,238 (10 mg/kg) contained only the same amount of noradrenaline as did resting samples. Neither drug increased the noradrenaline content of resting samples. These compounds act, therefore, like xylocholine by abolishing the release of transmitter substance during sympathetic nerve stimulation.

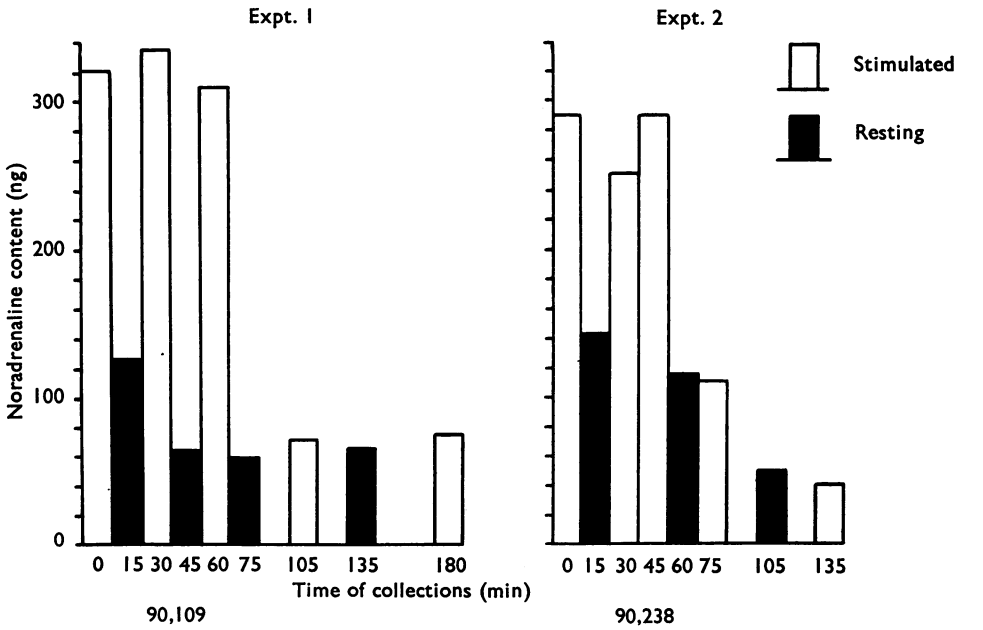



Fig. 7. Noradrenaline contents of blood samples collected from the splenic veins of cats anaesthetized with chloralose. The filled columns represent resting samples collected for periods of 20 sec and the open columns samples collected during stimulation (50 shocks/sec) of the splenic nerves for 15 sec and for the following 5 sec. The abscissae show the times at which the samples were collected and the ordinates the total amount of noradrenaline (as ng of base) in each sample. Experiment No. 1 shows the effect of 15 mg/kg of SK&F 90,109 and experiment No. 2 the effect of 10 mg/kg of SK&F 90,238. Both drugs inhibited the release into the venous blood of noradrenaline when the splenic nerves were stimulated.

*Effect of SK&F 90,109 and 90,238 on the release of catechol amines from the adrenal glands.* Experiments were done with cats whose intestines had been removed and kidneys denervated. Blood pressure responses to stimulating the left splanchnic nerves and to injections of adrenaline were recorded. Immediately after injecting 20 mg/kg of SK&F 90,238, which caused a large and prolonged rise in blood pressure, responses to splanchnic nerve stimulation and to adrenaline were greatly reduced. However, 15 to 30 min later splanchnic nerve stimulation produced as large a rise in blood pressure as before the drug, and the responses to adrenaline were enhanced. SK&F 90,109 did not affect the responses to splanchnic nerve stimulation although it greatly increased the responses to adrenaline.

In some experiments the right superior cervical ganglion was removed, the contractions of both nictitating membranes were recorded, and the adrenal glands were stimulated by injecting about 500 $\mu$g of tetramethylammonium iodide. The right nictitating membrane responded with a slow contraction, and the left with a spiky contraction followed by a slow one. SK&F 90,238, which itself caused a marked and long-lasting contraction of both nictitating membranes, quickly abolished the spiky contraction but not the slow ones. Thus SK&F 90,238 readily abolished the stimulant effect of tetramethylammonium iodide on sympathetic ganglia but not on the adrenal glands.

*Effect of SK&F 90,109 and 90,238 on the noradrenaline content of heart tissues.* The amounts of noradrenaline in the hearts of male Wistar rats were determined after subcutaneous injections of SK&F 90,109 or 90,238 (50 mg/kg). The noradrenaline was extracted by the method of Shore & Olin (1958) and estimated spectrophotofluorimetrically. The results are summarized in Table 1. This shows that these drugs depleted heart tissues of noradrenaline only to a small extent compared with the effects of guanethidine and reserpine.

TABLE 1

EFFECT OF SK&F 90,109 AND 90,238 ON TISSUE NORADRENALINE

Noradrenaline contents (percentage of control values) of rat hearts following subcutaneous injection of SK&F 90,109, SK&F 90,238 or guanethidine or the interperitoneal injection of reserpine. Two animals were used for each estimation

| Drug and dose | Noradrenaline content (% of control) after | | |
|---|---|---|---|
| | 4 hr | 12 hr | 24 hr |
| SK&F 90,109 (50 mg/kg) | 81 | 68 | 74 |
| SK&F 90,238 (50 mg/kg) | 83 | 69 | 81 |
| Guanethidine (15 mg/kg) | — | — | 6 |
| Reserpine (1 mg/kg) | — | — | 1 |

*Effects of SK&F 90,109 and 90,238 on axonal conduction.* Action potentials were recorded from electrodes on the postganglionic trunk of the cervical sympathetic nerve in response to single shocks applied to the preganglionic nerve. Injection of SK&F 90,109 or 90,238 (10 mg/kg) abolished immediately the postganglionic action potentials. This effect was short lived and 5 min later the

action potentials were at control level and remained so for at least 1 hr. The transient suppression was probably due to brief ganglionic blockade.

Postganglionic action potentials picked up from the splenic nerves close to the spleen in response to distal stimulation were unchanged after the injection of 20 mg/kg of SK&F 90,109. These drugs do not, therefore, impair transmission along adrenergic nerve trunks.

*Other actions of SK&F 90,109 and 90,238.* Mice were given these drugs orally and observed for changes in appearance, behaviour and activity. Large doses (1.6 g/kg) caused some general depression. SK&F 90,109 in doses above 400 mg/kg caused a very large and prolonged mydriasis.

Given to cats, orally or subcutaneously, SK&F 90,109 in doses up to 160 mg/kg produced little change in behaviour apart from some lethargy. However, intravenous doses higher than 20 mg/kg resulted in death from respiratory failure.

Injected into spinal cats, or into cats anaesthetized with chloralose, SK&F 90,109 reduced the contractions of the gastrocnemius and soleus muscles elicited by stimulating the sciatic nerve. The drug was about forty-times less active than tubocurarine and, like it, was more active on the gastrocnemius than on the soleus muscle.

TABLE 2

ACUTE TOXICITY OF SK&F 90,109 AND 90,238

LD50 values (mg/kg) of SK&F 90,109 and 90,238 in albino mice. Limits of error ($P=0{\cdot}05$) are given in parentheses. I.v., intravenous; s.c., subcutaneous

| Sex | Route of administration | LD50s (mg/kg) of SK&F 90,109 | LD50s (mg/kg) of SK&F 90,238 |
|---|---|---|---|
| Male | I.v. | 16·6 (15·4–17·9) | 16·8 (14·6–19·3) |
| Female | I.v. | 17·2 (14·9–19·8) | 17·3 (15·5–19·3) |
| Male | S.c. | 283 (240–332) | 407 (361–458) |
| Female | S.c. | 261 (206–331) | 437 (383–499) |
| Male | Oral | 1,360 (1,050–1,500) | 2,830 (1,590–5,000) |
| Female | Oral | 1,590 (1,140–2,200) | 2,680 (2,030–3,540) |

Tested on guinea-pig skin by the method of Bülbring & Wajda (1945), 1.0% solutions of SK&F 90,109 or 90,238 showed no local anaesthetic activity.

Neither drug in concentrations up to 100 $\mu$g/ml. contracted isolated lengths of guinea-pig ileum. SK&F 90,238 showed some antihistamine and antiacetylcholine action; SK&F 90,109 antagonized the effects of histamine, acetylcholine and dimethylphenylpiperazinium iodide.

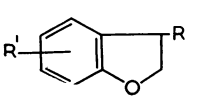
The intravenous, subcutaneous and oral LD50 values, determined in mice, are shown in Table 2.

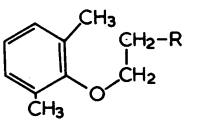
*Adrenergic-neurone blocking action in related compounds.* In Table 3 are shown the formulae, laboratory code numbers and the approximate adrenergic-neurone

TABLE 3

ADRENERGIC-NEURONE BLOCKING ACTIVITIES OF SOME COUMARANS AND CHOLINE XYLYL ETHERS

The numerals are the approximate relative activities (TM10, xylocholine=1.0) from weight for weight comparisons of the subcutaneous doses necessary to relax the nictitating membranes of cats to cover 30% of the lower eye lids. Zero activity indicates that the compound produced no effect at a dose of 25 mg/kg. The figures in parentheses are SK&F code numbers. [a] $ClO_4^-$; [b] $Br^-$; [c] HBr




| —R | R′=H | R′=7-$CH_3$ | R′=6-$CH_3$ | R′=5-$CH_3$ | R′=5-Cl | |
|---|---|---|---|---|---|---|
| $-NH_2$,HCl | | 0 (90,038) | 0 (90,396) | 0 (90,412) | | |
| $-N(CH_3)_2$,HCl | | 0 (90,039) | | | 0 (90,367) | 0 (TTM10)[c] |
| $-N^+(CH_3)_3I^-$ | 0·10 (90,102) | 0·20 (90,040) | 0 (90,411) | | 0 (90,371) | 1·0 (TM10)[b] |
| $-N^+(CH_3)_2(C_2H_5)I^-$ | | 0·30 (90,109) | 0 (90,430) | 0 (90,452)[a] | 0 (90,372) | 0·20 (90,011)[b] |
| $-N^+(CH_3)(C_2H_5)_2I^-$ | | 0·20 (90,158) | | | | 0·10 (90,010)[b] |
| $-NH.C(NH_2):NH,HNO_3$ | 0·15 (90,504) | 0·35 (90,238) | 0·10 (90,397) | 0·35 (90,427) | 0·65 (90,370) | |

blocking activities of the compounds tested. The activities quoted were determined in conscious cats by comparing, weight for weight, the subcutaneous doses that relaxed the nictitating membranes so that 30% of the eye was covered. Xylocholine was given an activity of 1.0. The drugs were also tested in anaesthetized cats (comparing the doses that abolished the contractions of the nictitating membranes to postganglionic sympathetic nerve stimulation), and in Finkleman preparations of rabbit ileum (comparing the concentrations that abolished the responses to periarterial nerve stimulation). The activities were of the same order by all three methods but the compounds in Table 3 which are classified as inactive, at the arbitrary dose level of 25 mg/kg, did produce, at higher doses, adrenergic-neurone blockade in anaesthetized cats.

It will be seen from Table 3 that the primary and tertiary amines were inactive. The simplest quaternary, coumaran-3-yltrimethylammonium iodide (SK&F 90,102), was slightly active ; its 6-methyl derivative was inactive but its 7-methyl derivative showed increased activity. Changing the quaternary group in the 7-methyl derivatives gave the most active quaternary compound, SK&F 90,109, which has the ethyldimethylammonium group. Retaining this group, but moving the 7-methyl substituent to either the 6- or the 5-position, abolished activity.

These trends may be compared to those found in the choline xylyl ether series. Compounds with the ring substitution pattern corresponding to the foregoing inactive quaternary compounds, namely, choline 2,5-xylyl ether bromide and choline 2,4-xylyl ether bromide (Brown & Hey, 1956) were also inactive as adrenergic-neurone blocking drugs (Fielden, unpublished). But in the choline xylyl ether series the trimethylammonium quaternary group conferred the greatest activity.

A different pattern emerged in the series of guanidines, all of which were active. The 7-methyl and 5-methyl derivatives were as active as SK&F 90,109, but, in contrast to the quaternary compounds, considerably increased activity was observed in *N*-(5-chlorocoumaran-3-yl)guanidine nitrate (SK&F 90,370), which was the most active compound in the series.

## DISCUSSION

SK&F 90,109 and 90,238 readily block for long periods the effects of stimulating adrenergic, but not cholinergic nerves, and do not generally antagonize the responses to adrenaline. Thus SK&F 90,109 and 90,238 resemble xylocholine (Exley, 1957), bretylium (Boura & Green, 1959) and, with some provisos, guanethidine (Maxwell, Plummer, Schneider, Povalski & Daniel, 1960).

It seems that the blocking action of SK&F 90,109 is more readily produced at some sites than at others. For example, considerably more of the drug is required to abolish the effects of hypogastric nerve stimulation than of other sympathetic nerves. Furthermore, the vasoconstriction in the hind-limb produced by stimulating the lumbar sympathetic nerves is blocked by doses of the drug that barely reduce the contractions of the nictitating membrane to sympathetic nerve stimulation. Such differences may have some practical significance, in so far as cardiovascular effects may be produced by doses of SK&F 90,109 that have little effect on some other sympathetically-innervated structures.

In the usual adrenergic-neurone blocking doses, SK&F 90,109 has little sympathomimetic action. It differs in this respect from bretylium whose more marked effects have been attributed to liberation of catechol amines from the effector organs (Gokhale, Gulati & Kelkar, 1963). The sympathomimetic actions of SK&F 90,238 on blood pressure and the nictitating membrane are much more marked and resemble those of guanethidine. But this action is not general, as there is no relaxation of the nonpregnant uterus and little tachycardia—although the latter may be masked by the antisympathomimetic action on the heart.

Experiments in conscious cats show, however, that in large doses SK&F 90,109 has a " sympathomimetic " action that antagonizes for some time the relaxation of the nictitating membranes resulting from loss of sympathetic tone. This was first revealed in experiments to determine dose/response curves 6 hr after giving the drug. Doses above 30 mg/kg caused progressively smaller responses, so that the curve reached a maximum and then decreased. Studies of time/response curves (see Fig. 3) showed that, as the dose of the drug was increased, the interval between drug administration and the appearance of the maximum effect also increased. This might be explained by the drug having two opposing actions but with different time/action relations. This was confirmed by experiments in which one nictitating membrane was relaxed by cutting the preganglionic cervical sympathetic nerve. Thereafter doses of SK&F 90,109 from 40 to 160 mg/kg retracted the relaxed membrane for some hours, but the innervated membrane did not relax until this effect had passed off. The contraction was not a result of ganglionic stimulation, as it was not prevented by treatment with hexamethonium and occurred even after recent removal of the superior cervical ganglion. It was not due to a muscarine-

like action on the muscle, as it was not reduced by atropine. A more likely cause seemed to be liberation of catechol amines. Experiments with phenoxybenzamine were, however, equivocal. Intravenous injections did not affect the action of SK&F 90,109 on the " decentralized " nictitating membranes of conscious cats, nor, however, were responses to adrenaline antagonized. Why phenoxybenzamine was without effect on the " decentralized " nictitating membranes of conscious cats we do not know.

Treatment with reserpine prevented this action of SK&F 90,109 and so suggested an action dependent on the noradrenaline stores in the effector organ. This was confirmed by experiments on cats in which one superior cervical ganglion was removed. Less than 24 hr after the operation an injection of SK&F 90,109 contracted the nictitating membrane, but had little effect when repeated 4 days later. Kirpeker, Cervoni & Furchgott (1962) showed that the noradrenaline content of the nictitating membrane was unchanged after cutting the preganglionic cervical sympathetic nerve, but was greatly, and permanently, reduced 24 to 48 hr after removing the superior cervical ganglion. Reserpine also depleted the nictitating membranes of their stores of noradrenaline.

Thus, large doses of SK&F 90,109 contract the nictitating membranes of conscious cats by an action dependent on stores of noradrenaline in the effector organ, and so delay the appearance of the effects of adrenergic-neurone blockade.

In conclusion, it is perhaps worth while to consider how the output of noradrenaline in response to adrenergic nerve stimulation may be prevented by drugs that act distally to the ganglion. The simplest situation is where the drug depletes the stores of noradrenaline at or in the region of the nerve endings, to such an extent that the effector organs show no response to nerve stimulation. This is how reserpine works (Muscholl & Vogt, 1958). Guanethidine, too, produces depletion in the long run, though its immediate action in blocking the liberation of noradrenaline is similar to that of xylocholine, bretylium (Cass & Spriggs, 1961) and the two drugs described in this paper.

The situation about drugs which do not act by depleting the stores of amines is more complex. The original view (Hey & Willey, 1954) about the mode of action of xylocholine was that, being a powerful local anaesthetic, it blocked axonal conduction in adrenergic fibres. Exley (1957, 1960) showed that neither xylocholine nor bretylium, in doses that were effective in preventing the liberation of noradrenaline on stimulation of the nerves, abolished conduction in the main trunk of the nerve. But Boura, Copp, Duncombe, Green & McCoubrey (1960) showed that bretylium, also a local anaesthetic, accumulated in adrenergic nerves, and would, at the terminal ramifications of these, reach concentrations sufficient to produce axonal block. Exley (1957) pointed out, however, that compounds related to xylocholine, and which were equally powerful as axonal blocking agents, did not produce the characteristic effect of xylocholine. Conversely, the drugs described here, SK&F 90,109 and SK&F 90,238, do not affect conduction in nerves and yet have a powerful xylocholine-like action on the effect of adrenergic nerve stimulation. Thus the facts that axonal blocking agents related to xylocholine fail to interfere with the liberation of noradrenaline on nerve stimulation, and that some drugs which are not axonal blocking

agents readily abolish the effects of adrenergic nerve stimulation, render suspect the local anaesthetic, or axonal-blocking, hypothesis.

What possibilities, then, remain ? Bain & Fielden (1957) suggested, from experiments on human chromaffin-cell tumours, that xylocholine might act by preventing the formation of noradrenaline from dopamine, by inhibiting dopamine-$\beta$-oxidase. There is strong presumptive evidence that the release of transmitter in response to nerve stimulation occurs only when the pool of preformed amine can be maintained constant, or nearly so, by the synthesis of fresh transmitter from dopamine, so that this hypothesis seems reasonable *a priori* (Bain, 1960). Hagen & Zebrowski (1962) also found that xylocholine inhibited dopamine-$\beta$-oxidase of human chromaffin-cell tumours, but that high concentrations were required. They apparently forgot that high concentrations of these drugs at their site of action were in fact achieved. On the other hand, preparations of dopamine-$\beta$-oxidase from bovine adrenal glands produced more, and not less, noradrenaline in the presence of xylocholine. That has been confirmed in this laboratory for xylocholine, SK&F 90,109 and 90,238 (A. L. Green, unpublished). But xylocholine and related drugs do not affect the response of the adrenal gland to stimulation, and so the situation here is evidently different from that at adrenergic nerve endings. The Bain-Fielden hypothesis must therefore stand or fall on the results of experiments giving direct evidence from adrenergic nerves themselves.

A further possibility derives from the Burn-Rand hypothesis, which regards acetylcholine as an intermediary in adrenergic nerve activity (Burn & Rand, 1959). The blocking actions of xylocholine, bretylium and guanethidine would, on this hypothesis, be accounted for by their blocking the effect of the liberated acetylcholine, and so preventing the subsequent liberation of noradrenaline. It is true that xylocholine, bretylium and guanethidine briefly antagonize the effect of acetycholine at autonomic ganglia and at neuromuscular junctions (Boura & Green, 1959 ; Dixit, Gulati & Gokhale, 1961 ; Exley, 1957 ; Willey, 1957). It is also true that drugs known to block autonomic ganglia and neuromuscular junctions have some adrenergic-neurone blocking activity (Burn & Froede, 1963). SK&F 90,109 and 90,238 also have these properties. But, even if one accepts the Burn-Rand hypothesis, it is difficult to see how prolonged adrenergic-neurone blockade can result from an action against acetylcholine, which, at least at the neuromuscular junction and the ganglionic synapse, is only short-lived.

It is therefore clear that the precise mode of action of SK&F 90,109 and 90,238, and indeed of the earlier drugs, has yet to be determined.

## CHEMICAL APPENDIX

The synthesis of each of these coumaran (2,3-dihydrobenzofuran) compounds started from the previously described coumaran-3-one oximes. Considerable improvements were effected in their preparation. The cyclization of 2-, 3- and 4-tolyloxyacetyl chloride to 7-methyl-, 6-methyl- and 5-methyl-coumaran-3-one, respectively, with aluminium chloride in benzene at 0° C was described by Higginbotham & Stephen (1920). We find that by using dichloromethane at $-10$ to $-15$° C increased yields of a cleaner product are obtained.

3-Tolyloxyacetyl chloride can cyclize in two ways to give either the 4-methyl- or the known 6-methyl-coumaran-3-one. Both products have now been isolated and characterized as the oximes. The latter is the major component.

The Friedel-Crafts cyclization of 4-chlorophenoxyacetyl chloride is known to be unsatisfactory (Kalinowski & Kalinowski, 1948). In experiments with 2-chlorophenoxyacetyl chloride the only product isolated was ω-(2-chlorophenoxy)acetophenone. 2-Chlorophenoxyacetic acid was recovered unchanged after 1 hr at 160° C in polyphosphoric acid.

An alternative synthesis of coumaran-3-ones has been described, which starts from a substituted salicylic acid (Armarego, 1960). By this method, methyl 5-chlorosalicylate was converted into ethyl 4-chloro-2-methoxycarbonylphenoxyacetate, which was subjected to the action of sodium ethoxide in benzene to give 5-chloro-2-ethoxycarbonylcoumaran-3-one. Hydrolysis and decarboxylation of this ester gave 5-chlorocoumaran-3-one in good yield. Since this work was done a similar series of reactions has been described by Schroeder, Corcoran, Holden & Mulligan (1962).

3-Aminocoumarans were obtained from the oximes by reduction with sodium amalgam in ethanolic acetic acid. The simplest method of preparing the 3-dimethylaminocoumarans is by the Eschweiler-Clarke procedure: when applied to 3-aminocoumaran and its 7-methyl- and 5-chloro-analogues a good yield was obtained. However, under the same reaction conditions 3-amino-5-methylcoumaran was degraded to 5-methylbenzofuran; 3-amino-6-methylcoumaran gave a low yield of the 3-dimethylamino-derivative, along with much 6-methylbenzofuran.

The synthesis of the other tertiary bases followed standard procedures. The 3-acetamidocoumarans were prepared in good yields by the action of an excess of acetic anhydride on the amine (sometimes in the presence of acetic acid or triethylamine). The 3-formamidocoumarans were prepared by the action of anhydrous chloral in chloroform on the amine (Blicke & Chi-Jung Lu, 1952). 6-Methyl-3-(*N*-methylacetamido)-coumaran was obtained in about 25% yield by the action of methyl iodide on the sodium salt of 3-acetamido-6-methylcoumaran (see Fones, 1949). Most of these amides were reduced directly with lithium aluminium hydride in refluxing tetrahydrofuran for between 5 and 12 hr.

The quaternization of the amines was straightforward, methyl iodide or ethyl iodide in methanol giving the quaternary iodide directly. Ethyldimethyl(5-methylcoumaran-3-yl)ammonium iodide could not be crystallized, but a crystalline perchlorate was obtained from the iodide, and, even better, from the *p*-toluenesulphonate prepared by reacting 3-ethylmethylamino-5-methylcoumaran and methyl *p*-toluenesulphonate at 60° C.

The guanidines in this series were all best prepared by reaction of the primary amine hydrochloride with an excess of cyanamide in boiling water. Isolation through the relatively insoluble bicarbonates was simple.

Table 4 shows most of the compounds prepared. Some of the intermediates were not characterized, as they were converted directly into the next product. Typical preparations follow.

TABLE 4

PROPERTIES OF COMPOUNDS NOT DESCRIBED IN THE TEXT

Good analyses for carbon, hydrogen, nitrogen and, where appropriate, halogen, were obtained for all compounds listed. * Double melting point; † in sealed tube; ‡, with decomposition

| SK&F No. | Formula I R′ | Formula I R | Formula | M.p. (°C) or b.p./pressure (°C/mm Hg) | Crystallized from |
|---|---|---|---|---|---|
| | H | $-N(CH_3)_2$,HCl | $C_{10}H_{14}ClNO$ | 169–172 | Ethanol/ether |
| 90,102 | H | $-N^+(CH_3)_3I^-$ | $C_{11}H_{16}INO$ | 173–174 and 260–263* | Methanol/ether |
| 90,504 | H | $-NH.C(NH_2):NH,HNO_3$ | $C_9H_{12}N_4O_4$ | 144–149 | Ethanol/ether |
| 90,038 | 7-$CH_3$ | $-NH_2$,HCl | $C_9H_{12}ClNO$ | 310–325‡ | Ethanol/ether |
| 90,039 | 7-$CH_3$ | $-N(CH_3)_2$,HCl | $C_{11}H_{16}ClNO$ | 218·5 B.p. of base is 127–128/21 | Ethanol |
| | 7-$CH_3$ | $-NH.CO.CH_3$ | $C_{11}H_{13}NO_2$ | 154·5 | Aqueous ethanol |
| | 7-$CH_3$ | $-NHC_2H_5$,HCl | $C_{11}H_{16}ClNO$ | 184·5 B.p. of base is 145–146/22 | Ethanol |
| | 7-$CH_3$ | $-N(C_2H_5)_2$,HI | $C_{13}H_{20}INO$ | 154·5‡ B.p. of base is 142/18 | Water |
| 90,040 | 7-$CH_3$ | $-N^+(CH_3)_3I^-$ | $C_{12}H_{18}INO$ | 256–259‡ | Methanol/ether |
| 90,109 | 7-$CH_3$ | $-N^+(CH_3)_2(C_2H_5)I^-$ | $C_{13}H_{20}INO$ | 146 | Ethanol |
| 90,158 | 7-$CH_3$ | $-N^+(CH_3)(C_2H_5)_2I^-$ | $C_{14}H_{22}INO$ | 136 | Methanol/ether |
| 90,238 | 7-$CH_3$ | $-NH.C(NH_2):NH,HNO_3,H_2O$ | $C_{10}H_{14}N_4O_4,H_2O$ | 139–140·5 | Water |
| 90,396 | 6-$CH_3$ | $-NH_2$,HCl | $C_9H_{12}ClNO$ | 238·5†‡ | Ethanol |
| | 6-$CH_3$ | $-NH.CO.CH_3$ | $C_{11}H_{13}NO_2$ | 189–190 | Ethanol |
| | 6-$CH_3$ | $-NHC_2H_5$,HCl | $C_{11}H_{16}ClNO$ | 211–212 B.p. of base is 104–105/2 | Ethanol/ether |
| 90,411 | 6-$CH_3$ | $-N^+(CH_3)_3I^-$ | $C_{12}H_{18}INO$ | 146–147‡ | Ethanol/ethyl acetate |
| 90,430 | 6-$CH_3$ | $-N^+(CH_3)_2(C_2H_5)I^-$ | $C_{13}H_{20}INO$ | 123–125 | Ethanol/ethyl acetate |
| 90,397 | 6-$CH_3$ | $-NH.C(NH_2):NH,HNO_3$ | $C_{10}H_{14}N_4O_4$ | 199–200‡ | Ethanol |
| | 5-$CH_3$ | $-NH.CO.CH_3$ | $C_{11}H_{13}NO_2$ | 171–172 | Benzene |
| | 5-$CH_3$ | $-NHC_2H_5$,HCl | $C_{11}H_6ClNO$ | 165–169 | Ethanol/ether |
| | 5-$CH_3$ | $-N(CH_3)(C_2H_5)$ | $C_{12}H_{17}NO$ | 92/0·6 | |
| 90,452 | 5-$CH_3$ | $-N^+(CH_3)_2(C_2H_5)ClO_4^-$ | $C_{13}H_{20}ClNO_5$ | 111–114 | Ethanol |
| 90,427 | 5-$CH_3$ | $-NH.C(NH_2):NH,HNO_3$ | $C_{10}H_{14}N_4O_4$ | 161–162 | Acetone |
| | 5-Cl | $-NH_2$,HCl | $C_8H_9Cl_2NO$ | About 300 (sublimes) | 6N-Hydrochloric acid |
| 90,367 | 5-Cl | $-N(CH_3)_2$,HCl | $C_{10}H_{13}Cl_2NO$ | 221–223 | Ethanol/ether |
| 90,371 | 5-Cl | $-N^+(CH_3)_3I^-$ | $C_{11}H_{15}ClINO$ | 183–185 and about 250*‡ | Ethanol |
| 90,372 | 5-Cl | $-N^+(CH_3)_2(C_2H_5)I^-$ | $C_{12}H_{17}ClINO$ | 151–152 | Isopropanol |
| 90,370 | 5-Cl | $-NH.C(NH_2):NH,HNO_3$ | $C_9H_{11}ClN_4O_4$ | 237·5–238·5‡ | Water |

*7-Methylcoumaran-3-one.* Aluminium chloride (428 g) in dichloromethane (1100 ml.) was stirred and protected from moisture. The solution was cooled to −15° C, and a solution of 2-tolyloxyacetyl chloride (538 g) (Higginbotham & Stephen, 1920) in dichloromethane (280 ml.) was added during 1 hr while the internal temperature was kept at −10 to −15° C. The mixture was stirred under these conditions for a further 2 hr, and then poured into a mixture of ice (3 kg) and 12 N-hydrochloric acid (700 ml.). The organic layer was separated, the aqueous phase was extracted with dichloromethane, and the combined organic solutions were evaporated. Steam-distillation of the residue, and addition of sodium chloride (2 kg) to the distillate (20 l.), precipitated the colourless crystalline, nearly pure product (244 g; 57%). Crystallization from absolute ethanol gave the pure ketone,

melting point 93 to 94° C; Higginbotham & Stephen (1920) record a melting point of 88° C.

*4- and 6-Methylcoumaran-3-one.* The yield of crude coumaran-3-ones from 3-tolyloxyacetyl chloride was 68%. This gave a mixture of oximes, which was separated into pure 6-methylcoumaran-3-one oxime, melting point 171 to 173° C [Higginbotham & Stephen (1920) record a melting point of 156° C; Fries & Finck (1908) record 165° C] and 4-methylcoumaran-3-one oxime, melting point 189 to 190° C. (Found: C, 66.26; H, 5.63; N, 8.64%. Calculated for $C_9H_9NO_2$: C, 66.23; H, 5.56; N, 8.58%.)

*Ethyl 4-chloro-2-methoxycarbonylphenoxyacetate.* Methyl 5-chlorosalicylate (31.8 g), ethyl bromoacetate (31.4 g) and anhydrous potassium carbonate (60.0 g) were heated in acetone (200 ml.) under reflux for 6 hr. The cold mixture was filtered and evaporated to an orange oil, which was dissolved in ether and extracted several times with 2 N-sodium hydroxide solution, washed with 10% aqueous sodium chloride solution, dried and evaporated to give a low melting point solid. Two crystallizations from ethanol gave the colourless di-ester (37.5 g; 81%), melting point 47 to 48° C. (Found: C, 52.88; H, 4.66; Cl, 13.30%. Calculated for $C_{12}H_{13}ClO_5$: C, 52.86; H, 4.80; Cl, 13.01%.)

*5-Chloro-2-ethoxycarbonylcoumaran-3-one.* The foregoing di-ester (952 g) in benzene (6 l.) was slowly added to a stirred suspension of sodium ethoxide (238 g) in benzene (2 l.) boiling under reflux and protected from moisture. After the addition, the mixture was kept under these conditions for 4 hr, cooled, treated with water (10 l.), and filtered to give a solid cake of the relatively insoluble sodium enolate of the keto-ester.

The aqueous layer of the filtrate was acidified with 6 N-hydrochloric acid to give a yellow solid, which was combined with the above sodium salt and suspended in hot ethanol (about 2.5 l.). After neutralizing with ethanolic hydrogen chloride and filtering, the hot solution deposited the keto-ester (565 g), melting point 134 to 138.5° C. A second crop gave 92 g more, melting point 130 to 137° C. The combined yield was 78%.

A sample crystallized from ethanol had melting point 139 to 140° C. (Found: C, 54.64; H, 3.71; Cl, 14.91%. Calculated for $C_{11}H_9ClO_4$: C, 54.89; H, 3.77; Cl, 14.73%.) Schroeder *et al.* (1962) record a melting point of 126 to 127° C.

*5-Chlorocoumaran-3-one oxime.* A 30% aqueous sodium hydroxide solution (540 ml.) was added during 30 min to the foregoing keto-ester (29.5 g) in boiling ethanol (300 ml.). After heating under reflux for a further 30 min, the mixture was acidified at about 40° C with 6 N-hydrochloric acid. After chilling to 0° C, the coumaranone, melting point 95 to 100° C, was collected. When crystallized twice from ethanol it had melting point 115 to 116° C. Schroeder *et al.* (1962) record a melting point of 114.5 to 116° C.

The crude coumaranone was converted into the oxime with hydroxylamine hydrochloride in boiling aqueous ethanolic sodium acetate to give colourless needles (12.5 g; 56% based on the keto-ester), melting point 188 to 191° C (decomposition), after crystallization from ethanol. (Found: C, 52.51; H, 3.35; Cl, 19.38; N, 7.50%.

Calculated for $C_8H_6ClNO_2$: C, 52.33; H, 3.29; Cl, 19.32; N, 7.63%.) Minton & Stephen (1922) record a melting point of 168° C.

*3-Amino-7-methylcoumaran.* 7-Methylcoumaran-3-one oxime (84 g) of melting point 155 to 157° C [Higginbotham & Stephen (1920) record a melting point of 148° C; Auwers (1916) records 152° C] was dissolved in a mixture of absolute ethanol (100 ml.) and glacial acetic acid (200 ml.) at about 65° C, and 5% sodium amalgam (1,600 g) was added during 4 hr, while the stirred mixture was maintained at 65° C. After a further 6 hr under these conditions, the mixture was diluted with water (2.7 l.) and made acid to Congo Red with 2 N-hydrochloric acid. After extraction with ether, the solution was basified and the amine was extracted with ether. Distillation of the extract gave the amine (35 g; 46%), boiling point 134° C (20 mm Hg).

*Other 3-aminocoumarans.* 6-Methylcoumaran-3-one oxime similarly gave 67% of 3-amino-6-methylcoumaran, melting point 41 to 43° C.

3-Amino-5-methylcoumaran, boiling point 110 to 111° C (3 mm Hg), was obtained in 53% yield; its hydrochloride had melting point 290 to 300° C (decomposition) [Stoermer & Barthelmes (1915) give a melting point of 230° C (decomposition)].

3-Amino-5-chlorocoumaran, boiling point 90° C (0.2 mm Hg), was obtained in 57% yield; it had a melting point of about 30° C.

*3-Dimethylamino-7-methylcoumaran.* The primary amine (16.2 g), 37% aqueous formaldehyde (19.5 ml) and 98% formic acid (23 ml.) were heated together on a steam-bath for 12 hr. After cooling, diluting with water and basifying, the tertiary amine was extracted with ether, and the extract was distilled to give the coumaran (13.1 g; 68%) boiling point 127 to 128° C (21 mm Hg).

*Other 3-dimethylaminocoumarans.* 3-Dimethylamino-6-methylcoumaran was obtained similarly in about 10% yield, calculated from the amount of quaternary methiodide produced by reacting the basic product with methyl iodide. Much neutral 6-methylbenzofuran was isolated.

5-Chloro-3-dimethylaminocoumaran, boiling point 84° C (0.4 mm Hg) was obtained similarly in 86% yield.

N-*(5-Chlorocoumaran-3-yl)guanidine nitrate.* 3-Amino-5-chlorocoumaran hydrochloride (7.7 g) and cyanamide (6.7 g.) were stirred for 24 hr in water (40 ml.) boiling under reflux. After chilling to 0° C for several hours, the solid was filtered off and washed with a little ice-cold water. Potassium bicarbonate (4.1 g) was added to the warmed combined filtrate and washings, and the resulting precipitate was collected, dissolved in boiling water (125 ml.) and treated with 8 N-nitric acid (5 ml.). The substituted guanidine nitrate, which crystallized on cooling, was dried at 60° C *in vacuo* and weighed 5.6 g, melting point 233 to 234° C (decomposition). Recrystallization from water raised the melting point to 237.5 to 238.5° C (decomposition).

N-*(Coumaran-3-yl)guanidine nitrate.* The unsubstituted *N*-(coumaran-3-yl)-guanidine nitrate was prepared in the same way. Its bicarbonate has been described

by Villere & Grinsteins (1958), who state that a temperature of 150° C (sealed tube) is required to cause the amine hydrochloride and cyanamide to react.

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