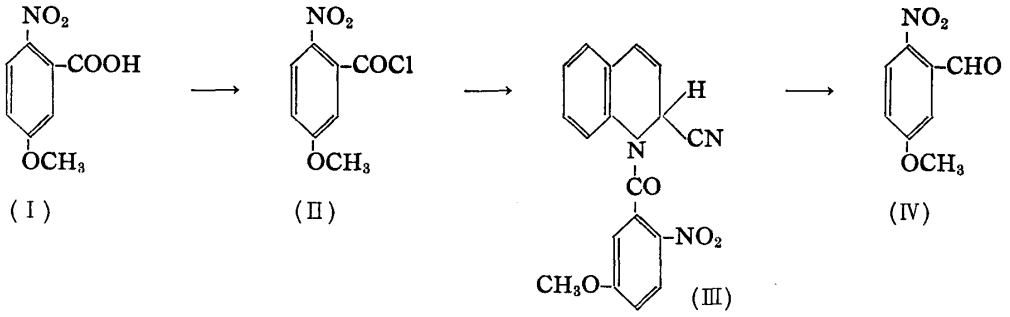
gen cyanide, and dehyd. benzene. The objective aldehyde was then obtained by hydrolysis of (III) with mineral acid. All the steps proceeded smoothly to give (IV) of satisfactory purity in good overall yield (approximately 62%, based on the starting acid).



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Experimental*2

2-Nitro-5-methoxybenzoyl Chloride (II)—A mixture of 4 g. of 2-nitro-5-methoxybenzoic acid (I)[3] and 20 cc. of $SOCl_2$ was warmed to 60° for 30 min. After removal of the excess $SOCl_2$ *in vacuo*, the residue was crystallized from petr. ether to yield 3.6 g. (82.2%) of colorless needles, m.p. 33~34°.

1-(2-Nitro-5-methoxybenzoyl)-1,2-dihydroquinaldonitrile (III)—To a solution of 1.5 cc. of anhyd. HCN in 7 g. of quinoline, protected from moisture and cooled to 0°, a solution of 5 g. of the chloride (II) in 5 cc. of dehyd. benzene was added. After 20 hr. at room temperature, the reaction mixture was diluted with benzene, washed successively with dil. H_2SO_4, $NaHCO_3$ solution, and water, and dried over Na_2SO_4. After evaporation of the solvent, the residue was crystallized from EtOH to give 7 g. (90.0%) of light yellow prisms, m.p. 153°. *Anal.* Calcd. for $C_{18}H_{13}O_4N_3$: C, 64.48; H, 3.91; N, 12.53. Found: C, 64.32; H, 4.19; N, 12.33.

2-Nitro-5-methoxybenzaldehyde (IV)—A mixture of 4 g. of the Reissert compound (III) and 100 cc. of 10*N* H_2SO_4 was boiled for 3 hr. After cool, the reaction mixture was extracted with Et_2O, the Et_2O layer was washed with $NaHCO_3$ solution and water, and dried over Na_2SO_4. Removal of the solvent left a yellow solid, which after recrystallization from dil. EtOH, afforded 1.8 g. (83.3%) of the aldehyde as colorless needles, m.p. 83°. A mixed m.p. determination with the authentic specimen obtained from the procedure of Smith, *et al.*,[2] showed no depression.

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*2 All m.p.s are uncorrected.

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Norio Sugimoto, Yoshio Kowa, and Ko Higaki*1; Shigeru Nakamura, and Shigeru Yasaka*2: Expectorant Effect and Clinical Efficacy of 1-Methyl-3-(di-2-thienylmethylene)piperidine Citrate (AT-327).

(*Osaka Research Laboratory, Tanabe Seiyaku Co., Ltd.*,*1 *and National Sanatorium, Toneyama Hospital**2)

It has already been reported[1] that 1-methyl-3-(di-2-thienylmethylene)piperidine citrate (hereinafter designated as AT-327) and its hydrochloride (hereinafter designated as AT-327-HCl) are powerful non-narcotic antitussive and numerous reports have been made on their clinical effect.[2,3] Majority of representative antitussives known to date did not

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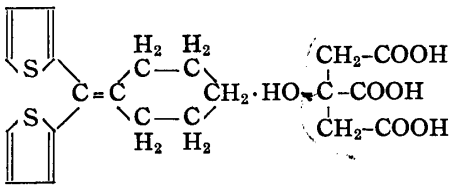
1) K. Higaki, G. Hayashi, T. Danno, Y. Kowa, N. Sugimoto: Yakugaku Kenkyu, **31**, 7(1959).
2) Y. Kase, *et al.*: This Bulletin, **7**, 372(1959).
3) M. Kimura: Rinsho-to-Kenkyu, **36**, 131(1959).

have expectorant effect or rather, they tended to inhibit secretion of tracheal mucus. Contrarily, AT-327 and AT-327-HCl were found to have expectorant effect as well, differing from the known antitussives, and some presumptions were made on their action mechanism, which are described herein.

Experimental Materials and Method



AT-327

Expectorant action was measured by the method of Sakuno[4] using rabbits and the potency was indicated by the following formula:

$$\frac{\text{Amount of dye excreted after sample administration}}{\text{Amount of dye excreted by non-treated normal rabbit}} = \text{Dye excretion index}$$

Expectorant effect is positive if this index is greater than 1 and negative if the index is equal to or less than 1.

Experimental Result

I. Excretion of Secretion by AT-327—Expectorant effect of AT-327 was compared with that of expectorants on the market, Senega syrup and apricot kernel water, and codeine phosphate and morphine hydrochloride.

Table I. Expectorant Action of Pharmaceutics

Substance	Manner of administrn.[a]	Dose (mg./kg.)	Dye excr. index[b]	Expectorant effect
AT-327	s. c.	16	1.64	+
AT-327-HCl	s. c.	16	1.75	+
AT-327-HCl	s. c.	32	2.13	+
Senega syrup J. P.	o.	2 cc./kg.	2.05	+
Apricot kernel water J. P.	o.	1 cc./kg.	1.50	+
Codeine phosphate	s. c.	32	0.92	−
Morphine hydrochloride	s. c.	16	0.88	−

a) s. c. = Subcutaneous injection. o. = Oral administration.
b) Average of 5 tests.

These results showed that there is an expectorant effect in AT-327, AT-327-HCl, Senega syrup, and apricot kernel water, but not in codeine phosphate and morphine hydrochloride, or rather, they inhibited secretion of tracheal mucus.

II. Action Time of AT-327—The expectorant action of AT-327 was observed periodically after subcutaneous administration of 30 mg./kg. dose. The action of AT-327 became apparent about 45 min. after its administration and mucus secretion reached the maximum after about 150 min. The activity was seen to continue even after passage of 390 min. (Fig. 1).

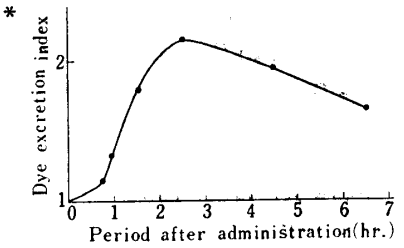


Fig. 1. Action Time of AT-327 (30 mg./kg. s.c.)

* Av. of 3 cases

4) N. Sakuno: Manshu Igaku Zasshi, **36**, 4(1942).

III. Amount of Dye Excretion by Changes in the Dosage of AT-327—Four dosage levels of 8, 16, 32, and 64 mg./kg. of AT-327 were administered by subcutaneous injection, the dye was injected 1 hr. later, and changes in the amount of the dye excreted were examined. With 8 mg./kg. of AT-327, there was hardly any acceleration of tracheal mucus secretion but increase of dosage to 16 mg./kg. effected 1.65-fold increase, and the secretion became twice the normal value when the dosage was increased to 32 mg./kg. Any further increase in dosage did not seem to effect any more increase in secretion (Fig. 2).

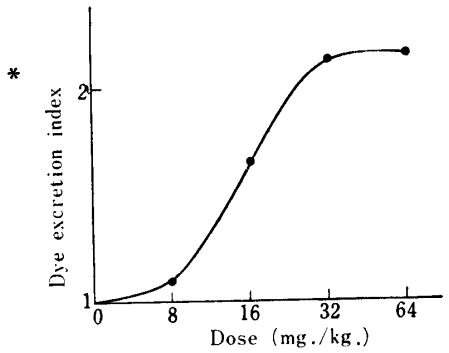


Fig. 2. Amount of Dye excreted with Variation in Dose

* Av. of 3 cases.

IV. Difference in the Amount of Dye Secretion according to Different Manner of Administration of AT-327—The amount of the dye excreted was examined after different mode of administration of AT-327 but increment of secretion of tracheal mucus was observed by any of the manner of administration.

Table II. Amount of Dye excreted after Different Manner of Administration of AT-327

Manner of administration	Dose administered (mg./kg.)	Dye excretion index[a)]
Subcutaneous injection	61.6	2.00
Intramuscular injection	61.6	2.17
Intravenous injection	11.6	1.66
Oral administration	173.4	2.30

q) Average of 3 tests; index 150 min. after administration of AT-327.

V. Action of Citric Acid—AT-327 is a citrate and there was some doubt that the expectorant effect might be partly due to the citric acid. Consequently, the action of citric acid was examined by subcutaneous injection of 25 or 50 mg./kg. of citric acid and the amount of dye excreted was observed 90 min. later. The dye excretion index was 0.97 after administration of 25 mg./kg. of citric acid and 0.92 after administration of 50 mg./kg. of the acid, showing that the acid had no expectorant action.

VI. Effect of Vagotomy on Dye Excretion by AT-327—The foregoing experimental results still did not show whether the action of AT-327 is central or not and therefore effect of vagotomy on the amount of tracheal mucus secretion was examined with emetine hydrochloride as the control. The amount of dye secretion was examined 150 min. after administration.

Table III. Effect of Vagotomy on Mucus Secretion

Substance	Dose (mg./kg.)	Dye excretion index			
		Oral		Subcutaneous	
		Non-treated	Vagotomized	Non-treated	Vagotomized
Emetine hydrochloride	4	1.52	0.95	1.65	0.97
AT-327	30	1.80	1.10	2.00	1.20

Both emetine hydrochloride and AT-327 increased secretion of tracheal mucus by either oral or subcutaneous administration but the secretion was hardly accelerated in the case of bilateral vagotomy.

VII. Clinical Effect—A total of 31 cases (10 males, 21 females) of pulmonary tuberculosis hospitalized in the National Sanatorium, Toneyama Hospital, in which the existing antitussive had been ineffective or had given very little effect, were given AT-327 for 10 days, during which administration of other drugs was stopped, and its effect was examined. There were only 5 cases in which this

substance was entirely ineffective, either as expectorant or antitussive, and there was practically no side effect.

Table IV. Expectorant and Antitussive Effect of AT-327 in Pulmonary Tuberculosis
(Daily dose : 90 mg., taken after each meal)

No. of cases	Effect	Marked effect	Effective	Somewhat effective	Ineffective
31	Expectoration	6	10	7	8
	Antitussive	9	8	5	9

Conclusion

1-Methyl-3-(di-2-thienylmethylene)piperidine citrate (AT-327) was found to possess expectorant effect and it also proved to be an excellent expectorant in clinical use. Some examinations were made on its action mechanism and a presumption was made.

1) Subcutaneous injection of 16 mg./kg. of AT-327 was found to give expectorant effect comparable to that of commercial expectorants.

2) The action of AT-327 became apparent about 60 minutes after subcutaneous injection of 30 mg./kg. of AT-327, the action reached the maximum about 150 minutes later, decreased gradually thereafter, but about one-half of the highest value remained even after 360 minutes.

3) Amount of tracheal mucus secretion increased in proportion to increasing dosage of AT-327 administered but any increased dosage above 32 mg./kg. by subcutaneous injection was not accompanied by increase in mucus secretion.

4) Secretion of tracheal mucus was accelerated by any mode of administration, whether subcutaneous, intramuscular, intravenous, or oral.

5) Accelerated secretion of tracheal mucus by AT-327 was not observed in vagotomized rabbit and, therefore, the action mechanism of AT-327 was deemed to be central.

6) Structurally, the principle of the action of AT-327 is considered to lie in 1-methyl-3-(di-2-thienylmethylene)piperidine.

7) There was no difference in the potency between the citrate and hydrochloride of this substance.

8) Examination of clinical effect was made in 31 cases of pulmonary tuberculosis and AT-327 was found to have marked expectorant and antitussive effect, with hardly any side effects.

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