temperatur stehengelassen, wobei das Addukt ausschied. Zu diesem Gemisch wurde eine Lösung von 1 g Na_2SO_3 in 30 cc 50-proz. EtOH gegeben, 3.5 Std. lang unter Rückfluss gekocht und dann filtriert. Das Filtrat wurde im Vakuum eingeengt, mit Wasser versetzt und mit Äther extrahiert. Der durch Eindampfen der Äther-Auszüge erhaltene Rückstand wurde aus MeOH umkristallisiert. Schmp. 160～163°. Ausbeute : 300 mg. Zur Analyse wurden sie bei 60° getrocknet. $C_{34}H_{52}O_4$-Ber.: C, 77.82; H, 9.99. Gef. : C, 77.57; H, 9.93. IR ν_{max}^{Nujol} cm^{-1} : 3520～3480 (OH); 1700 (Benzoyl-CO).

3β-Benzoyloxycholestan-14α,15β-diol—Zur Lösung von 5 g 3β-Benzoyloxycholest-14-en[9] in 100 ccm $CHCl_3$ wurde eine Lösung von Perbenzoesäure in $CHCl_3$ (1.2 Mols von aktiviertem Sauerstoff haltig) in der Kälte zugesetzt und die Reaktionslösung 40 Std. lang bei Zimmertemperatur stehengelassen. Die Lösung wurde erst mit 10-proz. NaOH-Lösung, dann mit Wasser gewaschen und getrocknet. Nach Eindampfen wurde der Rückstand aus Aceton umkristallisiert. Schmp. 152～154°. Das erhaltene 3β-Benzoyloxycholestan-14α,15α-epoxid wurde bei 40° im Hochvakuum getrocknet und analysiert. $C_{34}H_{50}O_3$-Ber. : C, 80.58; H, 9.95. Gef. : C, 80.55; H, 9.92.

0.5 g Epoxid wurden in 100 ccm Aceton gelöst, mit 10 ccm Wasser und 0.5 ccm 10-proz. $HClO_4$-Lösung versetzt und 40 Std. lang stehengelassen. Nach Zusetzen von Wasser wurde das Produkt mit Äther ausgezogen und aus Aceton umkristallisiert. Schmp. 202～205°. Ausbeute : 362 mg. Diol wurde bei 60° im Hochvakuum getrocknet und analysiert. $C_{34}H_{52}O_4$-Ber. : C, 77.82; H, 9.99. Gef. : C, 77.93; H, 9.96. IR ν_{max}^{Nujol} cm^{-1} : 3450 und 3550 (OH); 1690 (Benzoyl-CO).

Zusammenfassung

Progesteron (I) wurde durch Fermentierung mit *Syncephalastrum racemosum* Cohn in 7α,15β-Dihydroxyprogesteron (II) und Trihydroxyprogesteron (III) übergeführt. Das letztere konnte auch durch mikrobiologische Umwandlung von (II) mit gleichem Mikroorganismus erhalten werden. Damit wurden die Stellungen 7α und 15β für die zwei Hydroxy-Gruppen im Trihydroxylierungsprodukt gesichert. Für das dritte Hydroxyl wurde die 14α-Stellung vorgeschlagen und diese Zuordnung durch Vergleichung mit Cholestan-14α,15α-diol bzw. Cholestan-14α,15β-diol in Bezug auf der Verbrauchsgeschwindigkeit von Perjodsäure bzw. Bleitetraacetat gestützt.

(Eingegangen am 30. März, 1959)

UDC 615.722 : 547.822.3'732

73. Y. Kasé, T. Yuizono*1; T. Yamasaki, T. Yamada, S. Io, M. Tamiya, and I. Kondo*2 : A New Potent Non-narcotic Antitussive, 1-Methyl-3-di(2-thienyl)methylenepiperidine. Pharmacology and Clinical Efficacy.

(*Department of Chemico-Pharmacology, Faculty of Pharmacy, University of Kumamoto,**1 *and the Internal Clinic of the Kumamoto Central Hospital**2)

During the course of studies on the structure-activity relationship in antitussive effects of thienyl-alkylamino compounds,[1~5] it has been found that 1-methyl-3-di(2-thienyl)methylenepiperidine citrate (designated hereafter as AT-327), regarded as one of isomers produced by cyclization of $>C=C-C-N<^{CH_3}_{CH_3}$ chain of Iso-ohton or Ohton (3-dimethylamino-1,1-di(2-thienyl)but-1-ene), shows antitussive effect more potent than codeine in the dog and lower toxicity than Ohton. The fact that this compound is completely devoid of analgesic activity is the most favorable property for appraisal of the

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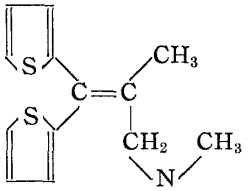
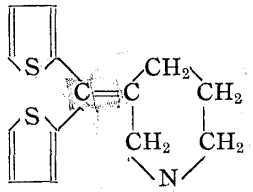
1) Y. Kasé : Japan. J. Pharmacol., **2**, 7(1952).
2) *Idem.* : This Bulletin, **2**, 298(1954).
3) *Idem.* : Japan. J. Pharmacol., **4**, 118(1955).
4) *Idem.* : *Ibid.*, **4**, 130(1955).
5) Y. Kasé, *et al.* : This Bulletin, **3**, 394(1955).

drug as an antitussive. These pharmacological properties of the drug in relation to those of other thienyl compounds were already reported by Kasé, *et al.*[6] in 1956. Furthermore, the drug never caused tolerance in antitussive effect even after continued daily administration of the drug for about 100 days and also seemed to establish no addiction liability as a result of observation of withdrawal symptoms induced by N-allylnormorphine (NANM) in the dog. The compound does not show any other undesirable pharmacological actions, such as depression of respiratory and cardiovascular systems in antitussive dose.

An excellent antitussive effect in various doses was also demonstrated in clinical trial and repeated administration of effective antitussive doses does not cause any untoward side-effects such as euphoria, craving, tolerance, or addiction. The details of this study will be published in the near future.



Iso-ohton



AT-327

Methods and Materials

1-Methyl-3-di(2-thienyl)methylenepiperidine citrate (AT-327) used for the experiment comes as needle crystals, m.p. 135°(decomp.), fairly soluble in water and stable in solution. The antitussive effect was evaluated by Kasé's method[4] in the unanesthetized dog and in the lightly anesthetized cat (pentobarbital-Na 20 mg./kg., intraperitoneal). Changes in respiration by coughing, caused by mechanical stimulation on the mucosa of tracheal bifurcation through a chronically built fistula, were recorded on a smoked paper. The antitussive effect of a certain drug was determined from the decrease in amplitude and frequency of cough curves, and from the duration of such effect. The dose necessary to depress coughing for 20~30 min. is taken as being effective, from which 50% antitussive dose (AtD_{50}) was calculated by Litchfield-Wilcoxon's method[7] ($p=0.05$). Analgesic activity was examined by a modification of the d'Amour-Smith's method[8] in mice; by pinching a pad of a dog with a hemostat. The acute toxicity was tested in mice (*dd*-strain, 20 ± 2 g.) and in dogs; LD_{50} was calculated by Litchfield-Wilcoxon's method. The methods used for testing other pharmacological actions will be described in each part concerned.

The clinical appraisal of antitussive effect and the observation of side-effects were carried out by oral application.

Results

I. Pharmacology*[1]

1) Antitussive effect: The comparison of antitussive effect of AT-327 with other control drugs is shown in Table I.

Compared by AtD_{50} of each drug, the antitussive effect of the drug is 1.62 times that of codeine in the dog and 0.90 times that of codeine in the cat. Therapeutic index (LD_{50}/AtD_{50}) is approximately equal to those of codeine and Ohton in the dog. At the dose level ranging from AtD_{50} to twice of AtD_{50}, it showed no respiratory depression, but rather a slight increase in respiratory rate in normal dogs.

The drug seems to depress coughing through direct action on cough center. The assumption was derived from the following results:

1) The dose of the drug necessary to depress coughing by administration into ver-

6) Y. Kasé, *et al.*: "Chemico-pharmacological studies on antitussive. Part 7." The Pharmacological Section, 9th Meeting of the Pharmaceutical Society of Japan, Fukuoka, April 8, 1956.
7) J.T. Litchfield, F. Wilcoxon: J. Pharmacol. Exptl. Therap., **96**, 99(1949).
8) F.E. d'Amour, D.L. Smith: *Ibid.*, **72**, 74(1941).

TABLE I.

Comparison of Antitussive Effect of AT-327 with Other Control Drugs (intravenous administration)

Drug		Dog (unanesthetized)				Cat (pentobarbitalized)		
		AtD_{50} mg./kg.	No. of dogs tested	Ratio of effect to codeine	LD_{50}/AtD_{50}	AtD_{50} mg./kg.	No. of cats tested	Ratio of effect to codeine
AT-327	Citrate	**2.79** (2.53～3.07)	24	**1.35**	**26.8**	**3.40** (2.88～4.02)	30	**0.74**
	Base	**1.64** (1.49～1.81)		**1.62**		**2.00** (1.19～2.37)		**0.90**
Ohton[a]	HCl-salt	0.85 (0.70～1.03)	30	4.42	27.2	1.49 (1.30～1.71)	20	1.70
	Base	0.75 (0.67～0.84)		3.53		1.31 (1.15～1.50)		1.36
Piperidino-ohton[b]	HCl-salt	0.27 (0.24～0.31)	36	13.93	61.6	0.64 (0.54～0.76)	25	3.95
	Base	0.24 (0.21～0.27)		11.04		0.57 (0.50～0.65)		3.14
Methadone (*d*+*l*)	HCl-salt	0.11 (0.08～0.14)	48	34.18	260.9	0.11 (0.08～0.15)	20	23.00
	Base	0.10 (0.07～0.13)		26.50		0.10 (0.07～0.14)		17.90
Morphine	HCl-salt[c]	0.41 (0.36～0.46)	138	9.17	426.8	0.25 (0.21～0.30)	20	10.12
	Base	0.31 (0.27～0.35)		8.55		0.19 (0.16～0.23)		9.42
Codeine	Phosphate[d]	**3.76** (3.38～4.19)	150	**1.00**	**26.6**	**2.53** (2.24～2.86)	20	**1.00**
	Base	**2.65** (2.38～2.96)		**1.00**		**1.79** (1.58～2.02)		**1.00**
Dextromethorphan	HBr-salt	10.2 (9.1～11.5)[e]	28	0.37	—	5.14 (4.64～5.70)	20	0.49
	Base	7.44 (6.61～8.37)[e]		0.36		3.77 (3.40～4.17)		0.48
Narcotine	HCl-salt[c]	22.3 (20.7～24.1)[f]	44	0.17	3.6	22.4 (19.4～25.9)	24	0.11
	Base	20.4 (18.9～22.0)		0.13		20.5 (17.7～23.7)		0.09
Tessalon[g]		2.98 (2.61～3.40)[f]	60	1.26	—	3.28 (2.99～3.60)	20	0.78
				0.89				0.55

a) 3-Dimethylamino-1,1-di(2-thienyl)but-1-ene.
b) 3-Piperidino-1,1-di(2-thienyl)but-1-ene.
c) Contains 1 mole H_2O.
d) Contains 3/2 mole H_2O.
e) Under pentobarbital-Na anesthesia.
f) Convulsion occurs.
g) Nonaëthyleneglycol *p*-butylaminobenzoate monomethyl ether.

tebral artery, common carotid artery, and cisterna cerebellomedullaris to the same degree as that by intravenous route was 1/10~1/6, 1/5~1/4, and 1/40~1/50 of intravenous dose, respectively.

2) Vagotomy for eliminating peripheral effect on stretch receptors in the lung hardly influenced antitussive effect of the drug in lightly pentobarbitalized dogs, in whicn coughing was induced by both electric stimulation of superior laryngeal nerve[1] and by mechanical stimulation of the mucosa of larynx.

3) The drug could depress centrally-induced cough responses[9,10] as well as reflex-induced ones in the cat fixed at a stereotaxic instrument and electrically stimulated on expiratory pace-maker area in the medulla.[11]

The most important and remarkable property of the drug is that the antitussive effect of the drug was never antagonized by NANM in the dog. Intravenous dose of 1.2 mg./kg. of Ohton depressed coughing completely for 35 mins., but such effect was immediately eliminated by intravenous administration of 0.12 mg./kg. of NANM in three dogs. In contrast to this, the effect of AT-327 was never eliminated by various doses of NANM corresponding to 1/2 to 1/10 dose of AT-327.

2) Toxicity : LD_{50} of AT-327 in mice and dogs are shown in Table II.

Table II. Comparison of LD_{50} of AT-327 with Other Control Drugs

Drug		Mouse (subcutaneous)		Dog (intravenous)	
		LD_{50} (mg./10 g.)	Ratio of dose to codeine	LD_{50} (mg./kg.)	Ratio of dose to codeine
AT-327	Citrate	**3.76** (3.29~4.30)	**0.51**	**74.8** (64.3~87.1)	**1.31**
	Base	**2.22** (1.94~2.53)	**0.61**	**44.1** (37.9~51.3)	**1.56**
Ohton	HCl-salt	1.37 (1.22~1.57)	1.39	23.1 (18.5~28.8)	4.23
	Base	1.21 (1.07~1.36)	1.12	20.3 (16.3~25.4)	3.40
Piperidino-ohton	HCl-salt	1.20 (1.10~1.31)	1.59	21.5 (19.1~24.2)	4.55
	Base	1.07 (0.98~1.17)	1.26	19.2 (17.1~21.5)	3.59
Methadone (*d*+*l*)	HCl-salt	0.31 (0.27~0.36)	6.16	28.7 (25.0~32.9)	3.41
	Base	0.28 (0.24~0.32)	4.82	25.7 (22.4~29.5)	2.69
Morphine	HCl-salt	3.54 (2.89~4.33)	0.54	175.0 (156.8~195.3)	0.56
	Base	2.69 (2.25~3.29)	0.50	132.8 (119.0~148.3)	0.52
Codeine	Phosphate	**1.91** (1.78~2.05)	**1.00**	**97.8** (76.8~124.5)	**1.00**
	Base	**1.35** (1.28~1.45)	**1.00**	**69.0** (54.2~87.8)	**1.00**
Dextromethorphan	HBr-salt	1.53 (1.34~1.74)	1.25	30.0[a]	3.26
	Base	1.12 (0.99~1.27)	1.21	22.0[a]	3.14
Narcotine	HCl-salt	19.8 (17.0~23.1)	0.10	80.0[a]	1.22
	Base	18.1 (15.6~21.1)	0.07	73.2[a]	1.06
Tessalon		1.0[b]	1.91 1.35	—	—

a) Minimal lethal dose. *b*) Cited from reference (13).

In mice, toxicity of the drug is about one-half that of codeine and less than that of Ohton or Dextromethorphan. In dogs, the drug is more toxic than codeine, but less toxic than other drugs such as Ohton, methadone, and Dextromethorphan by intravenous injection.

Toxic dose of the drug manifested general symptoms in mice due to the excitement of central nervous system such as restlessness, excitation, tremor, the Straub's tail phenomenon, jumping, and clonic convulsion. Death occurred from respiratory paralysis. The mice given the dose close to LD_{50} excreted red-tinted urine. No symptom was observed with AtD_{50} of the drug in the dog, but a few symptoms appeared at the dose above 5 mg./kg. (intravenous route). Anorexia and increase in respiratory rate were usual

9) H. Borison : Am. J. Physiol., **154**, 55(1948).
10) N. K. Chakravarty, *et al.* : J. Pharmacol. Exptl. Therap., **117**, 127(1956).
11) Y. Kasé, H. L. Borison : *Ibid.*, **122**, 215(1958).

symptoms, and salivation and muscular weakness were also observed in some of them. However, no vomiting or hypnosis was observed.

3) Analgesic activity : The drug showed no analgesic activity in mice even at the dose ranging from 1/2 to 2/3 of LD_{50} tested by d'Amour-Smith's method, though it clearly showed the Straub's tail phenomenon. No effect was recognized in the dog even with such large intravenous dose as 60 mg./kg. which is close to the lethal dose.

4) Tolerance and addiction liability : Continued daily dose of 5~6 mg./kg. of the drug by intravenous injection in 5 dogs for 62~65 days indicated that there was neither tendency of acute tolerance nor that of cumulative action in antitussive effect. The daily dose was increased to 10 mg./kg. and the dose was continued for 23 days, and then increased to 20 mg./kg. and continued for 14~16 days, but tolerance was not recognized, not only in antitussive effect but also in other side-effects such as anorexia and respiratory excitation. On the contrary, the continued daily intravenous administration of 2 mg./kg. of morphine or 10 mg./kg. of codeine indicated an establishment of complete tolerance within 4 or 2 weeks, respectively, not only in antitussive effect but also in other actions such as analgesia, salivation, and anorexia.

It is very difficult to find an addiction liability of a certain drug by animal experiment, but unmasking of withdrawal symptoms by the application of NANM to tolerant dogs seems to indicate the possibility for addiction liability. NANM (one-tenth dose of AT-327) was administered via intravenous or subcutaneous route into a group of 5 dogs (used for the above-described experiment to investigate tolerance of the drug for 99~104 days) at the last day of AT-327 administration. However, no withdrawal symptom was induced. In the case of morphine, the same procedure induced a marked withdrawal symptom in another group of six dogs. The symptoms were easily controlled by either morphine, methadone, or Ohton, but never by AT-327.

5) Action on cardiovascular and respiratory systems : The blood pressure of common carotid artery was recorded with Hg-manometer method and respiration was recorded with Marey's tambour via tracheal cannula in urethanized rabbits. Each dose level above 1 mg./kg. of intravenous dose of AT-327 caused a transitory fall of blood pressure (5~20 mm. Hg for 1~2 min.) and a slight increase in the amplitude of respiration simultaneously. Vagotomy could not eliminate the fall of blood pressure. Administration of a minute dose of the drug into vertebral artery also caused a slight fall. The cardiac movements recorded by cardiotambour concomitantly with respiration and blood pressure indicated that there was no inhibition on heart at the time of transitory fall of blood pressure. Movement of an excised rabbit heart was depressed and came to a standstill at the dilated position with 0.2 mg. of the drug tested by Langendorff's method, though there was no remarkable effect below the dose of 0.1 mg. AT-327 dilated the blood vessel of an excised rabbit ear in a concentration of 0.5×10^{-4} tested by Krakow-Pissemki's method, and this action seemed to be due to a direct action of the drug on the smooth muscle of blood vessel.

The cessation of respiration always preceded cardiac failure but, generally speaking, respiration was not so much affected as blood pressure even with doses near a lethal one.

6) Other pharmacological actions : The drug showed a local anesthetic activity about twice more potent than that of procaine, both in surface anesthesia examined by corneal reflex method in guinea pigs and in infiltration anesthesia tested by intracutaneous wheal method in guinea pigs. The drug showed no protective action against histamine asthma in guinea pigs tested by histamine aerosol method.[12] The tonus and pendular movements of excised small intestines of the rabbit were inhibited by the

12) J. W. E. Harisson, J. L. Ambrus, C. M. Ambrus : J. Am. Pharm. Assoc., Sci. Ed., **40**, 226(1951).

drug at the concentration of 10^{-5} tested by the Magnus method. The drug showed neurotropic and musculotropic spasmolytic effect in the rabbit small intestines, and furthermore, it showed an antihistaminic activity in guinea pig small intestines.

II. Clinical Efficacy[*2]

AT-327 was administered orally to 45 in- and out-patients suffering from severe coughing at the Internal Clinic of the Kumamoto Central Hospital. The classification of patients was 8 cases of far-advanced pulmonary tuberculosis, 28 cases of acute or chronic bronchitis, 2 cases of bronchial asthma, and 7 cases of common cold. Average daily effective dose of the drug medicated orally ranged from 80 to 100 mg. Some patients with severe coughing at night needed 20~30 mg. of the drug as a single medication before sleep. The classification of the effect is shown in Table III.

Table III.

Patients (No. tested)		Complete relief	Marked improvement	No relief
Pulmonary tuberculosis	(8)	1	5	2
Bronchitis	(28)	5	18	5
Bronchial asthma	(2)	0	0	2
Common cold	(7)	0	6	1
Total	(45)	6	29	10

The drug seems to be especially favorable for coughing with sputum, but it showed no effect on asthma. A prolonged medication of over 10 days in 13 patients (over 30 days in two out of them) showed neither establishment of tolerance, habituation, or cumulation, nor any untoward side-effect with the daily dose over 100 mg. In 11 out of 16 patients, whose symptoms did not improve with Dextromethorphan (60 mg.) or codeine phosphate (60 mg.), a marked improvement was observed with this drug. On the contrary, 4 out of 10 patients getting no relief from this drug showed favorable response to Dextromethorphan or codeine. It seems to be due to chemical changes (reduction?) of AT-327 in the digestive organs that the dose necessary for it to show a definite clinical efficacy is larger than that shown in the above-described pharmacological data in comparison with Dextromethorphan or codeine.

The authors wish to express their appreciation to Dr. N. Sugimoto of the Research Laboratory, Tanabe Seiyaku Co. Ltd., for generous supply of AT-327 and for his continued interst in this work.

Summary

1-Methyl-3-di(2-thienyl)methylenepiperidine citrate (AT-327) showed antitussive effect more potent than codeine in unanesthetized dogs and effect approximately equal to codeine in pentobarbitalized cats. The efficacy was also demonstrated in clinical trials with 45 patients. The antitussive activity seemed to be due to its direct action on the cough center *per se*. The drug did not show any analgesic activity, tendency of establishing tolerance, or indicate addiction liability, either in animal experiments or in clinical trials. The toxicity was found to be lower than that of codeine in the mouse. Other pharmacological properties of the drug were also discussed briefly.

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〔Added in proof〕 Nair and Haar published a paper[14] concerning a detailed clinical efficacy with an abstract of pharmacology of a similar compound, 1-ethyl-3-di(2-thienyl)-methylenepiperidine after the authors had reported[6] on antitussive effects and some other pharmacological properties of AT-327 (hydrochloride). Therefore, this study was carried out independently of them.

13) K. Bucher : Schweiz. Med. Wochschr., **86**, 10(1956).
14) K.G.S. Nair, H.O. Haar : New York State J. Med., **56**, 1773(1956).