Therefore, the link between the metal and the ligand is believed to be O-metal, and the above considerations favour the structure.

The simple cyclic form of the oxime acts as a unit in the complex formation, being stabilized in turn by the interaction with the metal halide. From another point of view, MCl_2 is thought to be sandwiched with two cyclic dimers of the oxime.

For the copper and manganese complexes, further study must be necessary to deduce their structure. But, of course, the precise structure for any of the complexes must not be decided until X-ray study will be carried out.

The authors are grateful to Prof. A. Nakahara for his valuable advices, and to Prof. M. Ishidate, Dr. K. Sakurai and Dr. Y. Tamura for discussions. The authors thank Prof. K. Takima for his interest. The authors are indebted to the members of analytical section of Shionogi Research Laboratory for microanalyses.

Summary

n-Butyraldoxime complexes of copper (II), nickel (II), cobalt (II), and manganese (II) were prepared by a direct reaction between the metal halides and the oxime. From their chemical and physical properties, a octahedral structure is deduced. Further, the infrared absorption specta suggest for the complexes that the oxime is co-ordinated through its oxygen atom in the simple cyclic structure of the oxime to the metal.

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80. Yoshio Ota, Nobuo Endo, and Midori Hirasawa : Antitussive Activity of Narcotine Derivatives.*1

(*Kowa Chemical Laboratories, Kowa Co., Ltd.**2)

The naturally occurring *l*-α-narcotine (Noscapine) is an alkaloid widely known as a non-narcotic antitussive agent. Takagi, *et al.*[1] studied the pharmacological properties

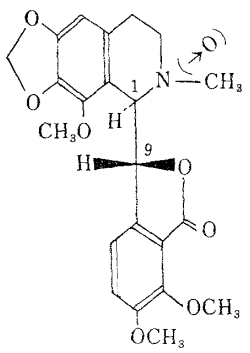

Chart 1. Chemical Structure of *l*-α-Narcotine and its N-Oxide (1*R*:9*S*)

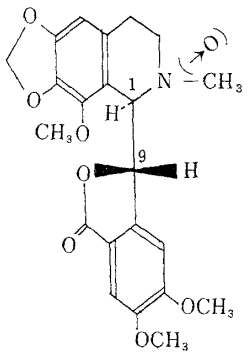

Chart 2. Chemical Structure of *l*-β-Narcotine and its N-Oxide (1*R*:9*R*)

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1) K. Takagi, H. Fukuda, *et al.* : Yakugaku Zasshi, **81**, 266 (1960).

of its N-oxide with special reference to its antitussive action. Pharmacological properties of its steric isomer, *l*-β-narcotine, and its N-oxide have not been reported to data. Recently, absolute configuration of *l*-α- and *l*-β-narcotine was determined by Ohta and others[2] of this laboratory and comparative examinations were made on the pharmacological properties of these compounds and their N-oxides.

Method

The narcotine derivatives were used as their hydrochlorides and they are often referred in the text as the test compound. Standard agents, except for dihydrocodeine phosphate, were also hydrochlorides.

Antitussive Action

Antitussive action was tested by both chemical and mechanical stimulation method. The animals used were male guinea pigs of 400~600 g. in body weight. The test was made by the method of Takagi, *et al.*,[3] with dihydrocodeine as the reference.

Test solution was injected intraperitoneally and the effective dose (ED_{50}) was calculated by the up-and-down method.

Analgesic Action

Pressure method : The modified pressure method of Takagi and Kameyama[4] was followed, using male mice of 15~20 g. in body weight, with 10 animals to each group, to examine the analgesic effect of the test compounds. The dose levels used were 200 mg./kg. of the test compound, 4 and 8 mg./kg. of morphine as the reference, and physiological saline solution was used as the control.

There were given subcutaneously.

Hot-plate method : The modified hot-plate method of Takagi and Kameyama[5] was used. Morphine, of which the analgesic action shows linear dose-action relationship, was given subcutaneously in doses of 3 and 6 mg./kg.

The test compound in a dose of 50 mg./kg. was given intraperitoneally 15 min. after the administration of morphine, and the presence or absence of increased action by the test compounds was examined 15 min. later.

When the jump reaction could not be observed within 30 sec., the animal was taken off the hot plate to avoid burns.

Inhibition of Intestinal Propulsion and Constipating Action

A group of 10 male mice, weighing around 15 g., was submitted to the test by the method of Takagi, and Fukuda.[6] Inhibition of intestinal propulsion was expressed as the percentage of the distance passed by $BaSO_4$ suspension through the intestine to the total length of the intestine.

Effect on constipation was expressed as the average length of time required for excretion of white feces after the administration of $BaSO_4$ suspension.

When this excretion required more than 10 hr., a mark $>$ was placed before the average value. The dose levels used were 300 mg./kg. of the test compound and 30 mg./kg. of morphine or dihydrocodeine as the reference (dihydrocodeine alone was used for the inhibition test). Physiological saline solution was used for the control. The drug solution was applied in the rate of 0.1 ml./10 g. body weight.

Action on Rectus Abdominis of Frog

Usual method was employed, using rectus abdominis muscle of frog. The contraction caused by the administration of 10^{-6} g./ml. of acetylcholine was taken as 100% and the contraction in the presence of test compound was expressed in percentage (a%) of the normal contraction. Acetylcholine was given 3 min. after addition of the test solution. The muscle contraction was observed for 4 min.

Inhibition by the test compound was expressed as $(100-a)$%.

Action on the Guinea Pig Smooth Muscle

Following the method of Takagi and Takayanagi,[7] excised tracheal muscle preparation was made and the action of the test compounds on the preparation was tested in the Magnus tube. When muscle contraction caused by 10^{-5} g./ml. of histamine became uniform, this contraction height was taken as a%.

2) M. Ohta, H. Tani, S. Morozumi, S. Kodaira : Tetrahedron Letters, in press.
3) K. Takagi, H. Fukuda, *et al.* : Yakugaku Zasshi, **80**, 1497 (1960).
4) K. Takagi, T. Kameyama : *Ibid.*, **78**, 553 (1958).
5) *Idem* : *Ibid.*, **77**, 871 (1957).
6) K. Takagi, H. Fukuda : *Ibid.*, **80**, 1501 (1960).
7) K. Takagi, I. Takayanagi : This Bulletin, **6**, 716 (1958).

Relaxing action of tracheal muscle by the test compounds was examined for 4 minutes before application of histamine and relaxation (in mm.) by 10^{-8} g./ml. of adrenaline used as the reference was taken as 1. Ratio of the degree of relaxation (in mm.) by the test compounds at each dosage level to that of adrenaline was calculated. Histamine was then applied to examine its inhibition by the test compound and the degree of this inhibition was expressed as $(100-a)\%$.

Action on Rabbit Respiration and Blood Pressure

Rabbits weighing 2~3 kg. were anesthetized with 1.7 g./kg. of urethane. The carotid arterial pressure was recorded with a mercury manometer and the respiration was recorded with Marey's tambour.

Acute Toxicity

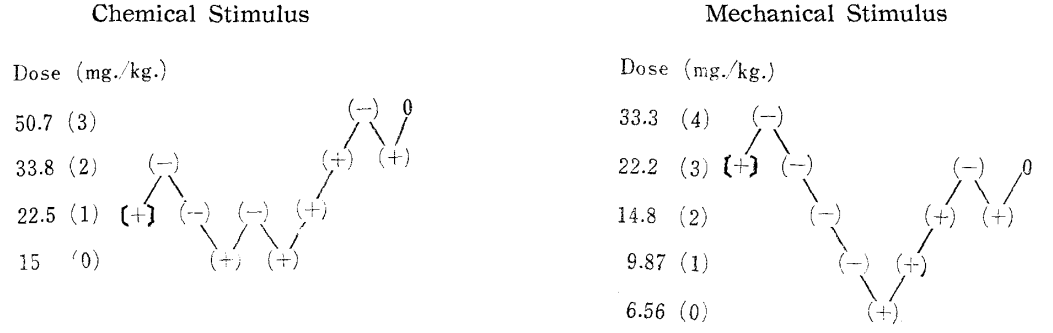
Groups of 10 male mice, weighing around 15 g., were used to test intraperitoneal and oral toxicity of the test compounds. Toxic symptoms and mortality were observed for 24 hr. to obtain the 50% convulsive dose (CD_{50}) and 50% lethal dose (LD_{50}).

Calculation followed the Litchfield-Wilcoxon method.

Results

Antitussive Action

Results obtained by the chemical and mechanical stimulation methods are given in Figs. 1~5 and in Table I. All the compounds tested were found to have antitussive effect.



(+) When cough occurred
(−) When no cough occurred

$$\frac{(0\times2)+(1\times3)+(2\times3)+(3\times2)}{10}=1.5$$

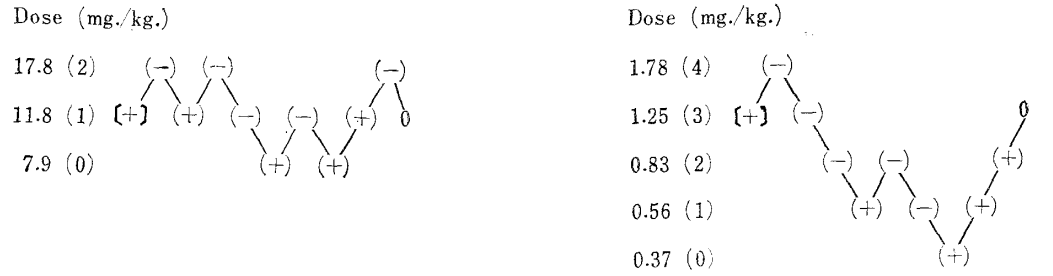
$\log ED_{50}=\log 22.5+0.5\times\log 1.5=1.44025$
$ED_{50}=27.6$ mg./kg.

(+) When cough occurred throughout 5 stimuli
(−) When no cough occurred even on one stimulus

$$\frac{(0\times1)+(1\times2)+(2\times3)+(3\times3)+(4\times1)}{10}=2.1$$

$\log ED_{50}=\log 14.86+0.1\times\log 1.5=1.18791$
$ED_{50}=15.41$ mg./kg.

Fig. 1. Estimation of Antitussive ED_{50} of *l*-α-Narcotine in Guinea Pig



$$\frac{(0\times2)+(1\times5)+(2\times3)}{10}=1.1$$

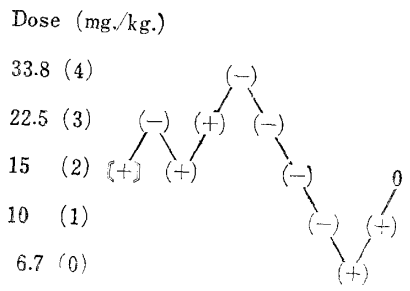
$\log ED_{50}=\log 11.8+0.1\times\log 1.5=1.08951$
$ED_{50}=12.3$ mg./kg.

$$\frac{(0\times1)+(1\times3)+(2\times3)+(3\times2)+(4\times1)}{10}=1.9$$

$\log ED_{50}=\log 0.56+0.9\times\log 1.5=\bar{1}.90668$
$ED_{50}=0.81$ mg./kg.

Fig. 2. Estimation of Antitussive ED_{50} of *l*-β-Narcotine in Guinea Pig

Chemical Stimulus

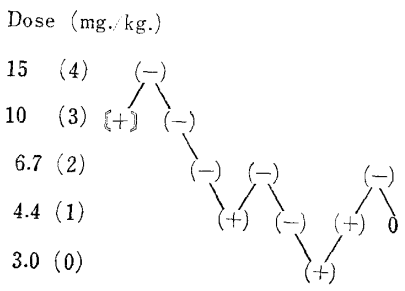


(+) When cough occurred
(−) When no cough occurred

$$\frac{(0\times1)+(1\times2)+(2\times3)+(3\times3)+(4\times1)}{10}=2.1$$

$\log ED_{50}=\log 15+0.1\times\log 1.5=1.19371$
$ED_{50}=15.7$ mg./kg.

Mechanical Stimulus

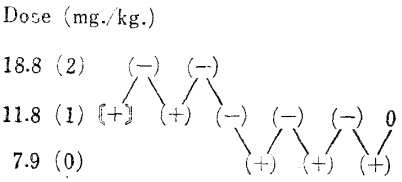


(+) When cough occurred throughout 5 stimuli
(−) When no cough occurred even on one stimulus

$$\frac{(0\times1)+(1\times4)+(2\times3)+(3\times1)+(4\times1)}{10}=1.7$$

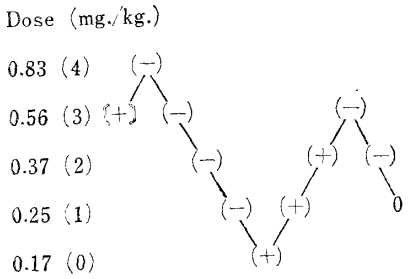
$\log ED_{50}=\log 4.4+0.7\times\log 1.5=0.76677$
$ED_{50}=5.7$ mg./kg.

Fig. 3. Estimation of Antitussive ED_{50} of *l*-α-Narcotine N-Oxide in Guinea Pig



$$\frac{(0\times3)+(1\times5)+(2\times2)}{10}=0.9$$

$\log ED_{50}=\log 7.9+0.9\times\log 1.5=1.05609$
$ED_{50}=11.4$ mg./kg.

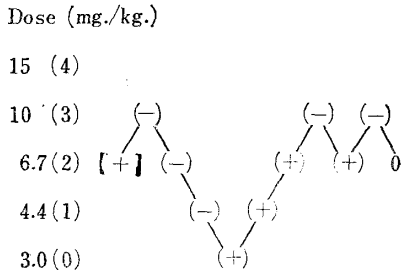


$$\frac{(0\times1)+(1\times3)+(2\times3)+(3\times3)+(4\times1)}{10}=1.9$$

$\log ED_{50}=\log 0.25+0.9\times\log 1.5=\bar{1}.55639$
$ED_{50}=0.36$ mg./kg.

Fig. 4. Estimation of Antitussive ED_{50} of *l*-β-Narcotine N-Oxide in Guinea Pig

Chemical Stimulus

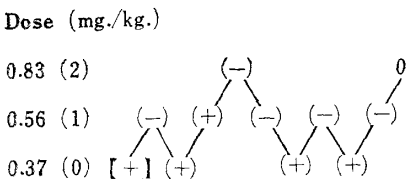


(+) When cough occurred
(−) When no cough occurred

$$\frac{(0\times1)+(1\times2)+(2\times4)+(3\times3)}{10}=1.9$$

$\log ED_{50}=\log 4.4+0.9\times\log 1.5=0.80199$
$ED_{50}=6.3$ mg./kg.

Mechanical Stimulus



(+) When cough occurred throughout 5 stimuli
(−) When no cough occurred even on one stimulus

$$\frac{(0\times2)+(1\times5)+(2\times2)}{10}=0.9$$

$\log ED_{50}=\log 0.37+0.9\times\log 1.5=\bar{1}.72669$
$ED_{50}=0.53$ mg./kg.

Fig. 5. Estimation of Antitussive ED_{50} of Dihydrocodeine in Guinea Pig

Table I. Antitussive Effects of *l*-α- and *l*-β-Narcotine and their N-Oxides on Guinea Pig by Chemical and Mechanical Stimulating Methods
ED_{50} (mg./kg., i.p.)

Compound	Chemical stimulus	Mechanical stimulus
l-α-Narcotine	27.6	15.4
l-β-Narcotine	12.3	0.8
l-α-Narcotine N-Oxide	15.7	5.8
l-β-Narcotine N-Oxide	11.4	0.4
Dihydrocodeine	6.3	0.5

As reported by Takagi, *et al.*,[1] *l*-α-narcotine N-oxide was found to be more effective than its original salt, and we found also that the steric isomers of the original salt, *i.e.*, *l*-β-narcotines had stronger action than *l*-α-narcotines.

l-β-Narcotine N-oxide also had a greater effect than its original salt and its action was stronger than that of dihydrocodeine by the mechanical stimulation method. The cause of the slight differences between the ED_{50} values by the chemical and the mechanical stimulation methods is still obscure despite further observation, but the appearance of the effect was approximately the same by either method. Since it was demonstrated that narcotine derivatives are a promising antitussive, we conducted examinations on their other pharmacological properties.

Analgesic Action

Pressure method: Mean of pain threshold values at intervals before and after administration of test compounds were calculated. Ten animals were used in each group. The data are shown in Figs. 6~9. The test compounds did not show any elevation of the pain threshold value despite a large dose like 200 mg./kg.
Hot-plate method: Total average of jump-reaction time for each dosage level was plotted and shown in Fig. 10.

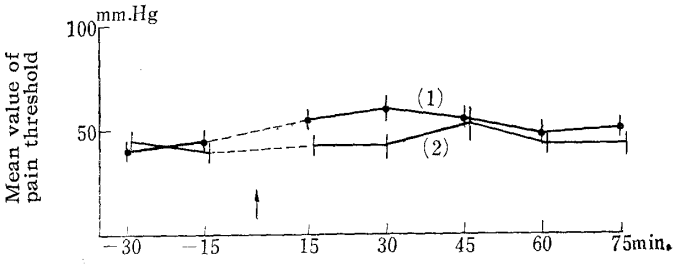


Fig. 6. Influence of *l*-α-Narcotine and its N-Oxide on Pain Threshold in Mice (200 mg./kg., s.c.)
(1): *l*-α-Narcotine (2): *l*-α-Narcotine N-oxide

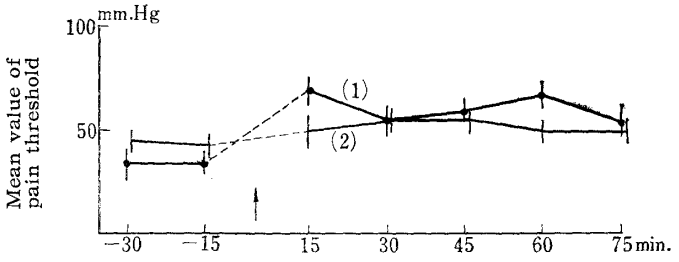


Fig. 7. Influence of *l*-β-Narcotine and its N-Oxide on Pain Threshold in Mice (200 mg./kg., s.c.)
(1): *l*-β-Narcotine (2): *l*-β-Narcotine N-oxide

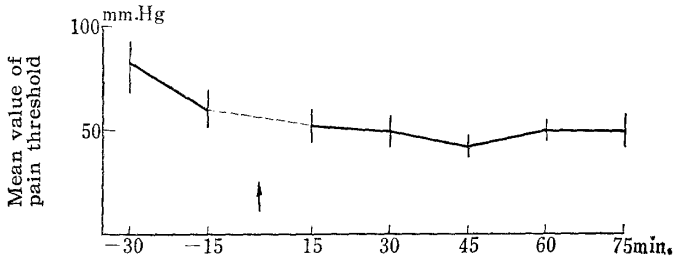


Fig. 8. Influence of Saline on Pain Threshold in Mice (0.1 ml./10 g., s.c.)

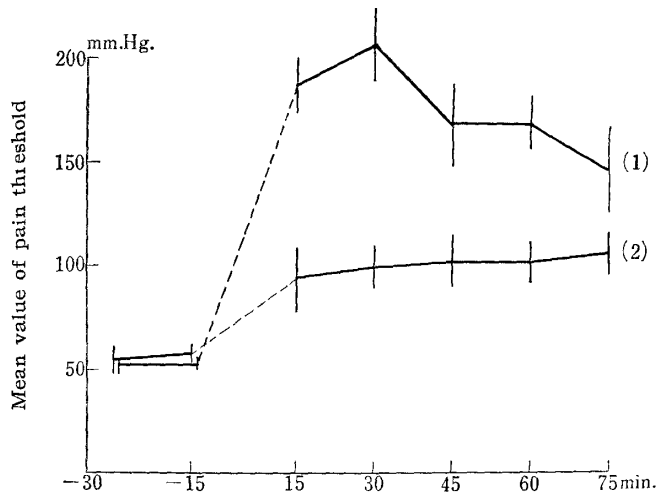


Fig. 9. Influence of Morphine on Pain Threshold in Mice (4 and 8 mg./kg., s.c.)
(1): 8 mg./kg. of morphine (2): 4 mg./kg. of morphine

The morphine group combined with each test compound showed no difference from the analgesic effect of morphine alone and augmentation of analgesic effect of morphine by the combination with test compounds could not be observed.

This differs from the report of Tanaka[8] that the analgesic action of morphine was potentiated by *l*-α-narcotine, using the Haffner method.

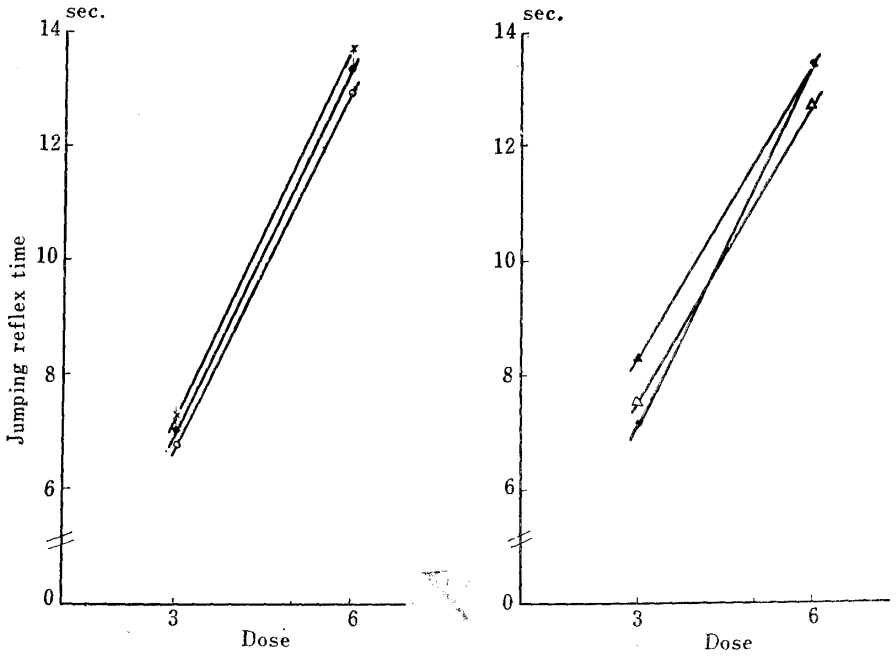


Fig. 10. Influence on Pain Threshold Elevating Effect of Morphine (3 and 6 mg./kg., s. c.) in Combination with Each of *l*-α- and *l*-β-Narcotine, and their N-Oxides (equally 50 mg./kg., i. p.)

×—× *l*-α-Narcotine combined group
○—○ *l*-α-Narcotine N-oxide combined group
△—△ *l*-β-Narcotine combined group
▲—▲ *l*-β-Narcotine N-oxide combined group
●—● Single morphine group

Effect on Rabbit Respiration and Blood Pressure

Results of these tests are given in Fig. 11. Blood pressure fell about 15~20 mm. Hg by the administration of 5 mg./kg. of *l*-α- or *l*-β-narcotine and a slight inhibition of respiration was observed. The corresponding N-oxides caused almost no change in respiration or blood pressure, and only a slight inhibition of respiration depth was observed by the administration of 10 mg./kg. of *l*-α-narcotine N-oxide. Marked fall in blood pressure was observed after administration of 10 mg./kg. of *l*-α- and *l*-β-narcotine, and a transitory apnoea and arhythmia (probably due to cardiac blockade) were observed after the administration of *l*-β-narcotine. It may be said from these results that *l*-α- and *l*-β-narcotine N-oxides have weaker effect on respiration and blood pressure than their original salts.

Inhibition of Intestinal Propulsion

As shown in Table II, the test compounds (300 mg./kg. p.o.) showed approximately the same results as that of physiological saline solution. On the other hand, significant difference was found in the mean propulsion rate of dihydrocodeine, although its dose was 1/10 that of the test compounds. Therefore, the test compounds have no action of inhibiting intestinal propulsion.

8) C. Tanaka : Folia Pharmacol. Japan., 58, 538 (1961).

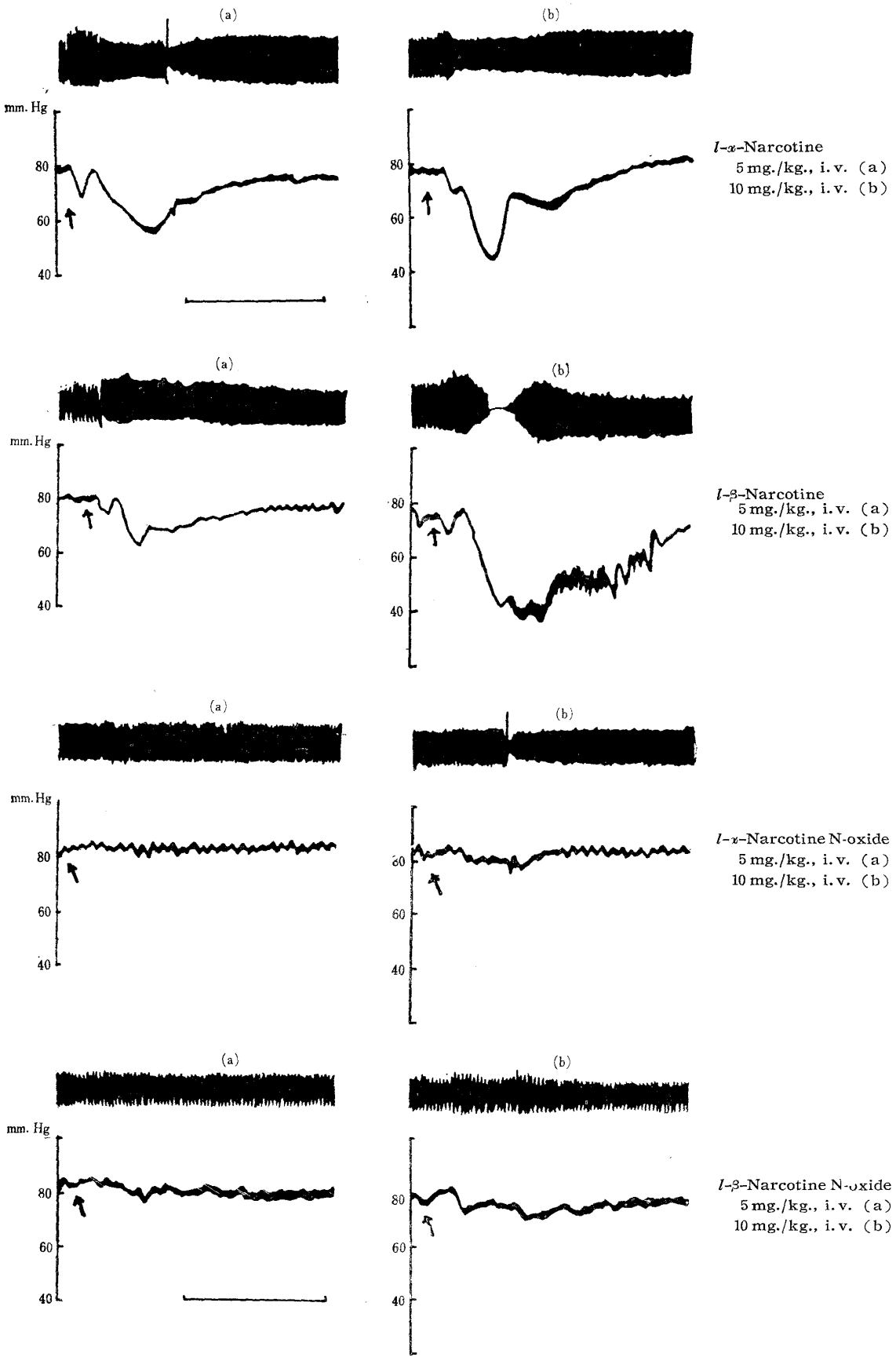


Fig. 11. Effect on Blood Pressure and Respiration

Table II. Percentage of Small Intestine traversed in 20 min. by Barium Sulfate Suspension after *l*-α- and *l*-β-Narcotine, and their N-Oxides

Compound	Dose (mg./kg., p.o.)	Mean ± S.E. (%)
l-α-Narcotine	300	53.2 4.39
l-β-Narcotine	300	37.9 4.31
l-α-Narcotine N-Oxide	300	48.2 3.41
l-β-Narcotine N-Oxide	300	44.3 2.90
Saline	—	48.6 4.29
Dihydrocodeine	30	a) 18.8 1.72

Average length of small intestine (60 mice): 41.7±1 cm. n=10 for each dose
a) $F_0=41.72>F_{18}=4.41$, $p=0.05$

Constipating Action

Table III gives the average period of time required for excretion. A significant difference was detected only in *l*-β-narcotine among the compounds tested. This indicates that *l*-β-narcotine has a slight constipative action, although it is not as strong as that of morphine and dihydrocodeine.

Table III. Times required for the First Excretion of White Feces after Oral Administration of Barium Sulfate Suspension

Compound	Dose (mg./kg., p.o.)	Excretion time (hr.) Mean ± S.E.
l-α-Narcotine	300	5.92 0.32
l-β-Narcotine	300	6.81 0.20
l-α-Narcotine N-Oxide	300	5.25 0.50
l-β-Narcotine N-Oxide	300	a) 5.97 0.15
Dihydrocodeine	30	>8.31
Morphine	30	>9.41
Saline	—	5.93 0.34

Compounds were given orally 1 hour before $BaSO_4$ suspension administration. n=10 for each dose
a) $F_0=4.9>F_{18}=4.41$, $p=0.05$

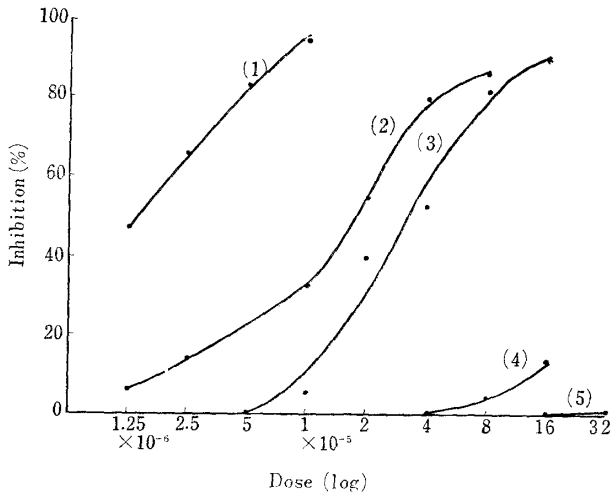


Fig. 12. Dose-Inhibition Curves of *l*-α- and *l*-β-Narcotine, and their N-Oxides, and *d*-Tubocuraline (*d*-Tc.) to 10^{-6} g./ml. Acetylcholine on the Rectus Abdominis Muscle of Frog

(1): *d*-Tc. (2): *l*-α-Narcotine
(3): *l*-β-Narcotine (4): *l*-β-Narcotine N-oxide
(5): *l*-β-Narcotine N-oxide

Action on Rectus Abdominis of Frog

As shown in Fig. 12, the anti-acetylcholine action of *l*-α-narcotine is stronger than that of *l*-β-narcotine but their action is weaker than that of *d*-tubocurarine.

Such inhibitory effect was not observed in any of their N-oxides and even *l*-β-narcotine N-oxide showed only a very weak inhibition in a high dosage.

Action on Guinea Pig Tracheal Muscle

Results shown in Fig. 13 indicate that both *l*-α- and *l*-β-narcotines have histamine antagonizing action. Inhibition of histamine contraction was ca. 60% in a concentration of 3.2×10^{-4} g./ml. of *l*-α-narcotine, and ca. 80% by *l*-β-compound. The same tendency

was also found in their relaxing action on the tracheal muscle. The N-oxides of both these compounds also showed these actions in a high dosage but the action of *l*-*β*-narcotine N-oxide was weaker.

Acute Toxicity

General toxic symptoms in mice by the administration of *l*-*α*- or *l*-*β*-narcotine were similar in either route of administration. In the convulsive dose, an excitation state, which was easily induced by a sound, appeared first, followed gradually by tonic convulsion or sometimes by clonic convulsion. This was followed by respiratory inhibition and collapse, resulting in death. Death seemed to be due to respiratory paralysis. In the narcotine N-oxides, there was no initial excitation, and majority of toxic symptoms appeared in association with cyanosis, and took relatively longer time for appearance. As shown in Table Ⅳ, CD_{50} was smaller by intraperitoneal administration than by oral route, except in the case of *l*-*β*-narcotine N-oxide, and especially so in *l*-*β*-narcotine N-oxide by either route but the time required for the appearance of toxicity was slow as stated above.

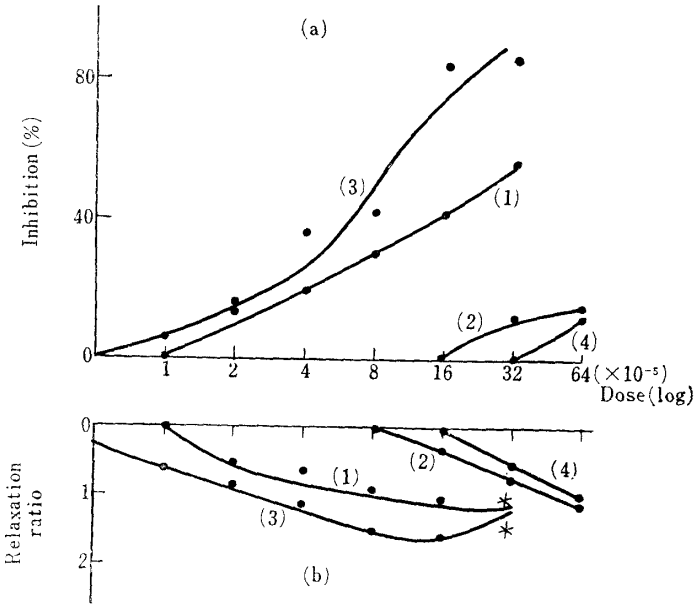


Fig. 13.

(a) : Dose-Inhibition curves
(b) : Dose-Relaxation ratio curves (R. ratio)
(1) : *l*-*α*-Narcotine (2) : *l*-*α*-Narcotime N-oxide
(3) : *l*-*β*-Narcotine (4) : *l*-*β*-Narcotine N-oxide
An asterisk shows turbidity of free bases in these doses.

Table Ⅳ. Convulsive Dose 50 (CD_{50}) and Lethal Dose 50 (LD_{50}) of *l*-*α*- and *l*-*β*-Narcotine, and their N-Oxides by Intraperitoneal and Oral Administration

Compound	CD_{50} (mg./kg., i.p.)	LD_{50} (mg./kg., i.p.)	CD_{50} (mg./kg., p.o.)	LD_{50} (mg./kg., p.o.)
l-*α*-Narcotine	480 (537.6～ 393.4)	960 (1219.2～ 755.9)	680 (897.6～ 515.2)	960 (1180.8～ 780.5)
l-*β*-Narcotine	90 (115.2～ 70.3)	255 (300.9～ 216.1)	580 (696 ～ 483.3)	840 (1075.2～ 656.3)
l-*α*-Narcotine N-Oxide	1100 (1243 ～ 973.5)	1175 (1316 ～1049.1)	1950 (2671.5～1423.4)	2500 (3325 ～1879.1)
l-*β*-Narcotine N-Oxide	1175 (1327.8～1039.8)	1250 (1387.5～1126.1)	820 (1049.6～ 640.6)	1250 (1712.5～ 912.4)

( )=95% fiducial limits

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Summary

Antitussive action and general pharmacological properties were comparatively examined in narcotine derivatives, especially the steric isomers, *i.e.*, *l*-*α*- and *l*-*β*-narcotine, and their N-oxides. *l*-*β*-Narcotine showed better antitussive effect than *l*-*α*-

narcotine by chemical and mechanical stimulation methods using guinea pigs. The N-oxides of both *l*-α- and *l*-β-narcotines had better action than thier original salts and the antitussive action of *l*-β-narcotine N-oxide was more effective than that of dihydrocodeine when tested by the mechanical stimulation method.

Both *l*-α- and *l*-β-narcotines showed some activity in tests of anti-acetylcholine action on frog rectus abdominis muscle, respiration and blood pressure of rabbits, relaxation and histamine antagonizing action on guinea pig smooth muscle, and acute toxicity of mice. These compounds had no effect of increasing the analgesic action in combination with morphine, inhibition of intestinal propulsion in mice, or constipative action. N-oxides of both *l*-α- and *l*-β-narcotines had weak or entirely no effect in these tests, except for a marked antitussive action and relaxation of tracheal muscle in comparatively high dosage.

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81. **Kazukichi Kato** : A New Color Reaction of Steroid with Anhydrous Aluminum Chloride and Anisaldehyde. I. A Colorimetric Determination of Ethylestrenol.

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Ethylestrenol (17α-ethyl-17β-hydroxyestr-4-ene) is an anabolic hormone widely used. There is no colorimetric method for this type of steroid, except that with concentrated sulfuric acid.[1] Sulfuric acid, however, is not a specific reagent for steroid as well known, and gives only a yellow color to ethylestrenol. In fact, in the assay of a preparation of this steroid it tends to give an error owing to simultaneous coloration of other inseparable components, for example, an antioxidant.

In Liebermann-Burchard's reaction[2,3] of this steroid, a violet color is immediately observed, but it fades so rapidly, that this reaction is unsuitable to the quantitative determination. Commonly used color reactions specific for functional groups of steroid are not successfully applied for ethylestrenol, because it has only one double bond, one ethyl and one hydroxyl group.

It gives no color in Kägi-Miescher's reaction,[4] in which steroid is heated with concentrated sulfuric acid and bromine or aromatic aldehyde, for this reaction is specific for the steroid having a secondary α-hydroxyl group at its 17-position.

In research for a more rational method of the assay, the combination of anhydrous aluminum chloride and anisaldehyde was found to be most suitable for this steroid, and therewith a new colorimetric determination was achieved.

Experimental

Material——Ethylestrenol : Ethylestrenol (N. V. Organon, Holland) was dried at 30° to constant weight under reduced pressure (5 mm. Hg). m.p. 94.6°. *Anal.* Calcd. for $C_{20}H_{32}O$: C, 83.33; H, 11.11. Found : C, 83.43; H, 11.09.

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