In a study of structure-activity relationship in antitussive agents, a working hypothesis has been presented that the introduction of a piperidino group into a compound showing any actions on the central nervous system, can produce antitussive activity if the activity has been latent, or strengthen it if such activity is already manifest (1, 2). In the previous study, antitussive activity of 2-allyloxy-4-chloro-N-(2-diethylaminoethyl) benzamide (264CE, Hexacol®) had been investigated and it was found that the activity of the drug was 1/4 to 1/3 as potent as that of codeine (3).

Therefore, in order to strengthen the antitussive activity, 2-allyloxy-4-chloro-N-(2-piperidinoethyl) benzamide (abbreviated as 264CP) in which a piperidino group was introduced in stead of a diethylamino group in the structure of 264CE was synthesized. Other 6 compounds including amino groups such as pyrrolidino, morpholino, piperazino, dimethylamino and primary amino groups in stead of a diethylamino group, and a trifluoromethyl group in stead of chlor (2-allyloxy-4-trifluoromethyl-N-(2-diethylaminoethyl) benzamide, 305CE) were also synthesized for testing their antitussive activities.

In the present experiment, the antitussive actions and other pharmacological actions of these 7 compounds, especially those of 264CP, have been compared with those of 264CE.

MATERIALS AND METHODS

The chemical structures of the test compounds are shown in Table 1. On use, they were dissolved in distilled water or physiological saline solution.

Toxicity: The male mice of dd-strain weighing 16–20 g were used and one group consisted of 6 mice and more than 5 groups were used for series of experiment. After the drugs were injected subcutaneously, toxic symptoms were observed. LD₅₀ and its fiducial limits (p=0.05) were calculated from lethality within 24 hours using the method of Litchfield-Wilcoxon (4). Mongrel dogs of both sexes weighing 6–10 kg were also used for the observation of toxic signs.

Antitussive activity: i) Mechanical stimulation. "Coughing dog and cat" methods
were used for this test. Dogs were used without anesthesia, while cats were lightly anesthetized with pentobarbital sodium (20-25 mg/kg, i.p.). Coughs were induced by mechanical stimulation of the mucosa at the tracheal bifurcation (5). Evaluation of the effect was made by the changes in amplitude and frequency of the tracing of coughs recorded on a smoked paper. When the amplitude and/or the frequency were decreased by more than 20% as compared with the pretreatment state and such a decrease lasted for more than 20 minutes, the effects were considered to be significant. More than 4 groups of animals were used and each group consisted of more than 5 animals. From the efficacy of the drug given intravenously, 50% antitussive dose (abbreviated as AtD₅₀) and its fiducial limits were calculated by the method of Litchfield-Wilcoxon (p=0.05). ii) Electrical stimulation of central stump of the superior laryngeal nerve (parameter of stimulus: frequency, 20 c.p.s.; duration, 1 msec; voltage, 0.48-3.8 V) in cats lightly anesthetized with pentobarbital (6). The antitussive effect was determined from the elevation of threshold voltage and the decrease in amplitude and frequency of coughs.

Analgesic action: In the male mice of dd-strain, weighing 16 to 18 g, analgesic activities of the drugs were examined with the methods of d'Amour-Smith (7) and Haffner (8).

Prolonging effects on anesthesia: To one group which consisted of 20 male mice of dd-strain weighing 14-18 g, a dose of 50 mg/kg of hexobarbital sodium was administered intraperitoneally, and the duration of anesthesia was measured as the interval between the loss and the recovery of the righting reflex. To other groups, each of which consisted of 10 mice, the test drugs were given intraperitoneally and, 20 minutes later, the same dose of hexobarbital was given in order to study how the duration of anesthesia was changed. The same experiment was done with codeine.

Local anesthetic actions: Surface anesthesia was examined by corneal reflex method in guinea pigs (9); infiltration anesthesia was examined by intracutaneous wheal method in guinea pigs (9).

Effects on the intestines: Influences on the transportation of intestinal contents were examined with the charcoal meal test in male mice of dd-strain weighing 16 to 20 g, after 20 hours' fasting. The compounds were administered intraperitoneally, and the 50% inhibitory dose was calculated by the method of Litchfield-Wilcoxon (p=0.05).

Effects on respiration and blood pressure: In dogs anesthetized with morphine and urethane (10 mg/kg + 1 g/kg, s.c.), blood pressure of the femoral artery was recorded with the routine mercury manometer method and the respiration was recorded with a Marey's tambour via tracheal cannula.

RESULTS AND DISCUSSION

I. Toxicity

1. Acute toxic symptoms in mice

In mice, the toxic symptoms caused by the compounds were similar to those of 264-CE, which showed ataxia, tremor, slight respiratory stimulation, stiffness of the extremities,
occasional elevation of the tail, vocalization, and occasional jumping with small doses, and convulsions with large doses.

Thus, toxic signs of all the compounds in mice are those of central excitation and death occurred with respiratory paralysis during or following tonic convulsions.

2. \( LD_{50} \)

The \( LD_{50} \) with its fiducial limits of the test compounds are shown in Table 1. The \( LD_{50} \) of codeine phosphate is also shown. In the 7 compounds, dimethylamino (264CD) and pyrrolidino (264CO) compounds were as toxic as 264CE. 264CP which showed the most potent antitussive activity (see the following) was approximately as toxic as codeine and 3/4 as toxic as 264CE. The other derivatives such as morpholino (264CM), piperazino (264CA), primary amino (264CF) compounds and 305CE were fairly weak and about a half as toxic as 264CE.

3. Toxicity in dogs

The lethal doses of the test compounds in dogs are shown in Table 1. With intravenous doses ranging from 10 to 50 mg/kg of the compounds, licking, salivation and clonic convulsions occurred during or immediately after the injection. These toxic signs were observed in common in all of the compounds tested. In dogs, 264CO was the most toxic and followed by 305CE. They are 1.3 to 1.7 times as toxic as 264CE. 264CP, 264CD and 264CA were approximately as toxic as 264CE and 2 times as toxic as codeine. While, 264CM and 264CF were 1/3 and 1/2 as toxic as 264CE.

**Table 1. Toxicity in mice and dogs.**

| \( R_1 \) | \( R_2 \) | \( LD_{50} \) | Ratio | \( LD \) |
|---------|---------|---------|-------|-------|
| 264CE   | \( C_2H_5 \) | Cl       | 155 (120-201) | 1 | 1.23 | 50 |
| 264CP   | \( C_2H_5 \) | Cl       | 204 (170-245) | 0.76 | 0.94 | 50 |
| 264CM   | \( O \) | Cl       | 300 (220-410) | 0.51 | 0.64 | 150 |
| 264CA   | \( N \) | Cl       | 338 (270-474) | 0.43 | 0.53 | 60 |
| 264CO   | \( N \) | Cl       | 172 (139-211) | 0.90 | 1.11 | 30 |
| 264CD   | \( C_2H_5 \) | Cl       | 150 (79-285) | 1.03 | 1.27 | 50 |
| 264CF   | \( NH_3 \) | Cl       | 280 (225-347) | 0.55 | 0.68 | 90 |
| 305CE   | \( N(C_2H_5) \) | CF_3     | 249 (226-279) | 0.62 | 0.77 | 40 |
| Codeine | \( H_2PO_4 \) | Cl       | 191 (178-205) | 0.81 | 1 | 97.8 |

\( LD_{50} \): mg/kg s.c. in mice; \( LD \): mg/kg i.v. in dogs

Numerals in parenthesis show the fiducial limits (\( p \)-0.05)
I. Dogs

As we had expected before, piperidino compound, 264CP, showed the most potent antitussive action, and it was 4.5 times as potent as 264CE and approximately as potent as codeine (see, Table 2). Fig. 1 shows the antitussive effects of 264CE and 264CP. An intravenous dose of 10.7 mg/kg of 264CE produced 30% decrease in the amplitude of cough and duration of the effect lasted for 20 minutes. Convulsions followed by tremor were observed with this dose of 264CE. On the other hand, 2.49 mg/kg of 264CP was enough to produce the same effect as that produced by 10.7 mg/kg of 264CE and no convulsion but a slight tremor was observed.

264CO (pyrrolidino compound), and 305CE also showed considerably potent antitussive actions. They were 2 to 3 times as potent as 264CE and 1/2 to 2/3 as potent as codeine. 264CM, 264CD and 264CA were approximately as potent as 264CE in their antitussive actions, while 264CF was ineffective with intravenous doses up to 30 mg/kg.

2. Cats

In cats, as shown in dogs, antitussive activity of 264CP was superior to those of any other compounds, though the activity in cats was less pronounced than that in dogs. It is 2 times as potent as 264CE and 2/5 to 1/2 as potent as codeine. With an exception of 264CF, other compounds were approximately as potent as 264CE and 1/5 to 1/4 as potent as codeine. 264CF was ineffective with the doses up to 30 mg/kg.

3. Safety margin

On intravenous injection to dogs, safety margin (LD/ATD₉₀) of 264CP, which showed the most potent antitussive action, was calculated to be about 21, and it was 4.6 times larger than that of 264CE and approximately a half that of codeine. The safety margins of the other compounds except for 264CF which showed no antitussive action in the doses...
TABLE 2. 50% antitussive dose.

| Compounds | "Coughing dog and cat" method | Domenjou's method |
|-----------|-------------------------------|-------------------|
|           | Dog mg/kg i.v. | Cat mg/kg i.v. | Cat mg/kg i.v. |
| 264CE     | 10.8 (8.5-13.8) | 9.8 (8.2-11.7) | 9.6 (8.0-11.5) |
| 264CP     | 2.4 (2.1-2.8) | 4.8 (3.7-6.2) | 5.2 (4.4-6.1) |
| 264CM     | 9.6 (8.5-10.8) | 9.8 (8.1-11.9) | 8.4 (6.8-10.8) |
| 264CA     | 9.0 (8.0-10.1) | 9.5 (7.9-11.4) | 10.2 (9.1-11.8) |
| 264CO     | 3.4 (2.8-4.2) | 8.2 (7.3-8.3) | 8.2 (7.0-9.7) |
| 264CD     | 9.3 (7.4-11.6) | 9.5 (7.9-11.4) | 9.8 (8.9-10.8) |
| 264CF     | >30             | >30              | >30              |
| 305CE     | 4.5 (4.0-5.1) | 7.5 (6.3-8.9) | 8.2 (7.5-9.0) |
| Codeine H₃PO₄| 2.5 (2.3-2.7) | 2.5 (2.2-2.8) | 2.0 (1.8-2.2) |

Domenjou's method: Coughs were induced by electrical stimulation of the superior laryngeal nerve. Dogs were used without anesthesia and cats were lightly pentobarbitalized.

used, were 1.2 to 3.5 times larger than that of 264CE.

III. Actions on the CNS

1. Analgesic action

The 50% analgesic doses (AD₅₀) of 264CP, 264CO, 264CE and codeine examined by the method of d'Amour-Smith were 13 mg/kg, 16 mg/kg, 30 mg/kg and 18 mg/kg, respectively. Thus, the analgesic actions of 264CP and 264CO which showed potent antitussive actions, were 2.3 and 1.9 times as potent as that of 264CE and slightly stronger than that of codeine. The other 5 compounds such as 264CA, 264CM, 264CD, 264CF and 305CE also showed analgesic actions and they were 1/3 to 1/2 as potent as that of codeine.

On the other hand, when the activities were examined by the method of Haffner, none of 7 compounds nor 264CE showed significant effect with the doses up to 1/2 LD₅₀. The AD₅₀ of codeine and morphine in this method were 69 mg/kg and 12 mg/kg, respectively.

2. Prolonging action on anesthesia

In mice, a significant prolongation of the duration of anesthesia was obtained with an intraperitoneal dose of 20 mg/kg of the compounds except for 264CD and 264CF.

Though codeine as well as 264CD and 264CF prolonged the duration of anesthesia, the differences were insignificant at the level of p=0.05. The prolonging action of 264CP was prominent and much stronger than those of 264CE and codeine.

TABLE 3. Analgesic action (d'Amour-Smith's method).

| Compounds | AD₅₀ mg/kg s.c. |
|-----------|-----------------|
| 264CE     | 30 (26-35)      |
| 264CP     | 13 (9-20)       |
| 264CM     | 27 (23-32)      |
| 264CA     | 41 (32-52)      |
| 264CO     | 16 (12-21)      |
| 264CD     | 35 (29-42)      |
| 264CF     | 58 (46-73)      |
| 305CE     | 33 (26-41)      |
| Codeine H₃PO₄ | 18 (14-22)   |
In guinea pigs, 264CP was 1.6 times as potent as procaine and 2.3 times as potent as 264CE in surface anesthesia, while it was 1.2 times as potent as procaine and 1.5 times as potent as 264CE in infiltration anesthesia. Any of other compounds were 0.8 to 1.4 times as potent as procaine both in surface and infiltration anesthesia.

IV. Local Anesthetic Action

In guinea pigs, 264CP was 1.6 times as potent as procaine and 2.3 times as potent as 264CE in surface anesthesia, while it was 1.2 times as potent as procaine and 1.5 times as potent as 264CE in infiltration anesthesia. Any of other compounds were 0.8 to 1.4 times as potent as procaine both in surface and infiltration anesthesia.

| Compounds | Dose mg/kg i.p. | No. of animals | Sleeping time in minutes | Probability |
|-----------|-----------------|----------------|--------------------------|-------------|
| Control   | —               | 20             | 11.1 (8.0–14.2)          | <0.05       |
| 264CE     | 20              | 10             | 22.1 (16.3–27.9)         | >0.05       |
| 264CP     | 1               | 10             | 13.4 (9.0–17.4)          | <0.05       |
|           | 5               | 10             | 25.0 (16.3–33.9)         | <0.05       |
|           | 20              | 10             | 31.1 (21.7–40.5)         | <0.05       |
| 264CM     | 20              | 10             | 21.3 (17.3–25.3)         | <0.05       |
| 264CA     | 20              | 10             | 38.1 (33.9–42.3)         | <0.05       |
| 264CO     | 20              | 10             | 24.6 (17.8–31.4)         | <0.05       |
| 264CD     | 20              | 10             | 14.0 (11.3–16.7)         | >0.05       |
| 264CF     | 20              | 10             | 14.9 (11.7–18.1)         | >0.05       |
| 305CE     | 20              | 10             | 20.7 (14.5–26.9)         | <0.05       |
| Codeine   | 5               | 10             | 14.5 (11.5–17.1)         | >0.05       |
| H₃PO₄     | 20              | 10             | 15.9 (11.0–20.8)         | >0.05       |

V. Action on the Intestines

On clinical application of narcotic antitussives such as codeine, constipation is one of the important side-effects. Therefore, the inhibitory actions of the compounds on the transportation of intestinal contents in mice were studied. The ED₅₀ of 264CP, 264CE and codeine were 18 mg/kg, 33 mg/kg and 7.6 mg/kg, respectively.

Thus, the inhibitory effect of 264CP was 1.8 times as much as that of 264CE but it was only 2/5 as potent as that of codeine. The inhibitory effects of other compounds were 1.2 to 1.5 times as potent as that of 264CE and 1/4 to 1/3 as potent as that of codeine.
In dogs anesthetized with morphine and urethane, intravenous doses more than 0.2 mg/kg of 264CE caused blood pressure fall and the degree of the fall depended upon the doses given.

Respiration showed an increase concomitantly with the fall of blood pressure. With a dose of 10 mg/kg of 264CE (=AtD₀ in unanesthetized dogs), the blood pressure which had been 120 to 130 mmHg fell by 90 mmHg immediately after the injection and returned to the preinjection level 90 minutes after the injection. Increases in amplitude and fre-

| Compd. | 0.2 | 0.5 | 1.0 | 2.0 | 5.0 | 10.0 | 20.0 |
|--------|-----|-----|-----|-----|-----|------|------|
|        | RESP. | n.c. | n.c. | +   | +   | +    | +    |
|        | B.P.  | 15   | 30   | 50  | 70  | 90   | dead |
| 264CE  | RESP. | n.c. | n.c. | n.c. | +   | +    | +    |
|        | B.P.  | 5    | 10   | 20  | 25  | 40   | 60   |
| 264CP  | RESP. | n.c. | 15   | 20  | 35  | 40   | 60   |
|        | B.P.  | 10   | 20   | 30  | 35  | 40   | 45   |
| 264CM  | RESP. | n.c. | n.c. | n.c. | +   | +    | +    |
|        | B.P.  | 10   | 20   | 30  | 35  | 40   | 45   |
| 264CA  | RESP. | n.c. | 10   | 15  | 20  | 35   | 60   |
|        | B.P.  | 10   | 20   | 30  | 35  | 40   | 45   |
| 264CD  | RESP. | n.c. | n.c. | n.c. | +   | +    | +    |
|        | B.P.  | 10   | 15   | 20  | 35  | 55   |
| 264CF  | RESP. | n.c. | 10   | 25  | 35  | 50   |
| 305CE  | RESP. | n.c. | 10   | 25  | 35  | 50   |
| Codeine| B.P.  | n.c. | 10   | 25  | 35  | 50   |

**FIG. 2. Actions on respiration and blood pressure.**

Morphine-urethanized dogs of both sexes weighing 6 to 10 kg. RESP., respiration; + ~ ++, degree of respiratory stimulation; -, respiratory depression; n.c., no change.

B.P., blood pressure; numerals, degree of blood pressure fall (mmHg).

The effects of 264CP, which showed the most potent antitussive action, are not so severe as those of 264CE.
quency of respiration accompanied with blood pressure fall and it lasted for 2 to 3 minutes.

The effects of the test compounds on respiration and blood pressure were qualitatively similar to, but less potent than, those of 264CE as shown in Fig. 2. They showed depressor action more potent than that of codeine, however, they did not cause respiratory depression which was commonly observed after injection of codeine. They caused short-lasting respiratory stimulation of reflex nature. With respect to 264CP, it caused blood pressure fall with doses more than 0.5 mg/kg. With 2 mg/kg (=AtD50 in unanesthetized dogs), blood pressure fell by 20 mmHg and respiration slightly increased. The effects both on respiration and blood pressure were of short duration, lasting for only 1 minute or so. Thus, in the antitussive dose, the effects of 264CP on respiration and blood pressure were not so strong as those of 264CE and rather mild.

SUMMARY

Antitussive activities and other related pharmacological activities of 7 congeners of 2-allyloxy-4-chloro-N-(2-diethylaminoethyl) benzamide (264CE, Hexacol®) have been investigated and compared with those of 264CE.

1. In 7 compounds, piperidino compound (264CP) showed the most potent antitussive action, and it was 4.5 times as potent as 264CE and approximately as potent as codeine in dogs. Pyrrolidino compound and 305CE, in which a trifluoromethyl group was introduced in stead of chlor, also showed considerably potent antitussive actions. They were 2 to 3 times as potent as 264CE and 1/2 to 2/3 as potent as codeine. Morpholino, dimethylamino and piperazino compounds were approximately as potent as 264CE, while primary amino compound was ineffective.

2. 264CP showed high safety margin, being about 21, and it was 4.6 times larger than that of 264CE and about a half that of codeine.

3. 264CP showed analgesic action more potent than those of codeine and 264CE when the action was examined by the method of d'Amour-Smith. The compound, however, showed no significant analgesic action when the action was examined by the method of Haffner.

4. 264CP prolonged the duration of anesthesia and the effect was much stronger than those of 264CE and codeine.

5. 264CP showed a local anesthetic action which is 1.2 to 1.6 times as potent as that of procaine and 1.5 to 2.3 times as potent as that of 264CE.

6. 264CP showed an inhibitory action on the transportation of intestinal contents. The effect was 1.8 times as potent as that of 264CE but it was only 2/5 as potent as that of codeine.

7. The effects of antitussive dose of 264CP on respiration and blood pressure were not so strong as those of 264CE and relatively mild.

8. 264CP was approximately as toxic as codeine but 3/4 as toxic as 264CE.

9. The present investigation offered an additional evidence supporting a hypothesis previously presented by the authors that a piperidino group strengthens antitussive activity.
It is concluded that 264CP showed much more potent antitussive action, weaker toxicity and milder adverse effects than 264CE.

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