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Synthesis and Pharmacological Activity of Sulfate Conjugates at 6-Position of *N*-Substituted Normorphine Derivatives

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Three pairs of *N*-substituted normorphine derivatives and the sulfate conjugates at the 6-position were tested for the analgesic and antagonistic activities and the development of physical dependence in mice. The compounds examined were nalorphine, nalorphine-6-sulfate (N-6-S), *N*-cyclopropylmethylnormorphine (CPN), *N*-cyclopropylmethylnormorphine-6-sulfate (C-6-S), *N*-dimethylallylnormorphine (DMN) and *N*-dimethylallylnormorphine-6-sulfate (D-6-S). The latter two pairs were newly synthesized. The analgesic activity of C-6-S and D-6-S was equipotent to that of CPN and DMN by the acetic acid writhing test on the s.c. injection, and the activity of N-6-S was about 2 times more potent than that of nalorphine. The antagonistic activity of N-6-S, C-6-S and D-6-S to morphine analgesia was higher than that of the parent compounds by the tail pinch test on i.c.v. injection. A withdrawal sign was seen in mice treated chronically with CPN, C-6-S and N-6-S by challenge with naloxone, whereas the mice treated with DMN, D-6-S and nalorphine showed no such sign. The effect of sulfation at the 6-position on the development of physical dependence was not well associated with the effect on agonistic and antagonistic activities.

Keywords opioid; *N*-cyclopropylmethylnormorphine; *N*-dimethylallylnormorphine; nalorphine; sulfate conjugate; analgesic activity; antagonistic activity; physical dependence

Previous works from our laboratory have shown that glucuronidation and sulfation of morphine at the 6-position enhance the analgesic activity.[1,2] Morphine-6-glucuronide is receiving considerable attention because of a higher level of this active metabolite than morphine in the blood of cancer patients with chronic dosings of morphine,[3,4] and the potential importance of glucuronide in morphine analgesia was appreciated.[5–7] The strong activity of morphine-6-glucuronide was unequivocally proved to be induced by the glucuronide itself.[8] A mixed agonist–antagonist, nalorphine was also modified in the same way and examined for its effect on agonistic and antagonistic activities and on the development of physical dependence.[9] The antagonistic activity of nalorphine to morphine analgesia was enhanced by glucuronidation and sulfation at the 6-position. These 6-conjugates of nalorphine were found to have potent dependence liability while the liability is low in nalorphine. Knoll *et al.* have reported the pharmacological profile following chemical modification at the 6-position of opiate analgesics with an azido group.[10–12] 6-Azidomorphine and 14-hydroxy-6-azidomorphine were shown to induce more potent analgesia than hydromorphone and oxymorphone, respectively. These derivatives were, further, reported to have a low dependence liability.

The experiments reported here were designed to examine the effects of 6-sulfation of *N*-substituted normorphine derivatives on the agonistic and antagonistic activities, and also on the development of physical dependence in mice. Compounds newly synthesized and tested for pharmacological activities were *N*-cyclopropylmethylnormorphine (CPN, **1**), its 6-sulfate (C-6-S, **3**), *N*-dimethylallylnormorphine (DMN, **2**) and its 6-sulfate (D-6-S, **4**), which are shown in Chart 1.

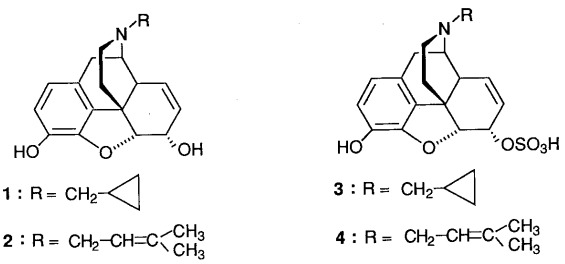


Chart 1

Chemistry Synthetic routes are shown in Chart 2. CPN (**1**) was prepared from normorphine (**5**) according to the method described by Gates and Monzka.[13] The starting material, normorphine (**5**), was synthesized from morphine in three steps according to the method reported by Rapoport.[14] DMN (**2**) was prepared from **5** according to the method described by Iijima *et al.*[15] **5** was alkylated with dimethylallyl bromide, and the resultant product (**2**) was purified by silica gel chromatography. C-6-S (**3**) and D-6-S (**4**) were synthesized following the method for morphine and nalorphine sulfates previously reported.[2,9] The intermediates **7** and **8** were used without further purification. Table I shows synthetic yields and melting points of the *N*-substituted normorphine derivatives newly synthesized in this paper.

Pharmacological Results The analgesic activity of *N*-substituted normorphine derivatives injected i.c. was assessed by the acetic acid writhing test in mice (Table II). The analgesic activity of nalorphine-6-sulfate (N-6-S) was about 2 times that of nalorphine as reported previously.[9] C-6-S and D-6-S, however, showed comparable analgesic activity to that of CPN and DMN, respectively. These compounds, except morphine, showed no analgesic activity when assessed by the tail pinch test in mice even at a high dose of 20 μmol/kg s.c. (data not shown).

The antagonistic activity of *N*-substituted normorphine derivatives and pentazocine to morphine analgesia was assessed by the tail pinch test in mice. All compounds, CPN, C-6-S, DMN, D-6-S, nalorphine, N-6-S, and pentazocine antagonized to the analgesic effect of morphine co-administered i.c.v. at a dose of 8 nmol/body (Table III). The sulfates, C-6-S, D-6-S and N-6-S showed more potent antagonistic activity than the parent compounds.

The effect on the development of physical dependence by 6-sulfation of *N*-substituted normorphine derivatives was examined in mice by 14 times s.c. administrations of the

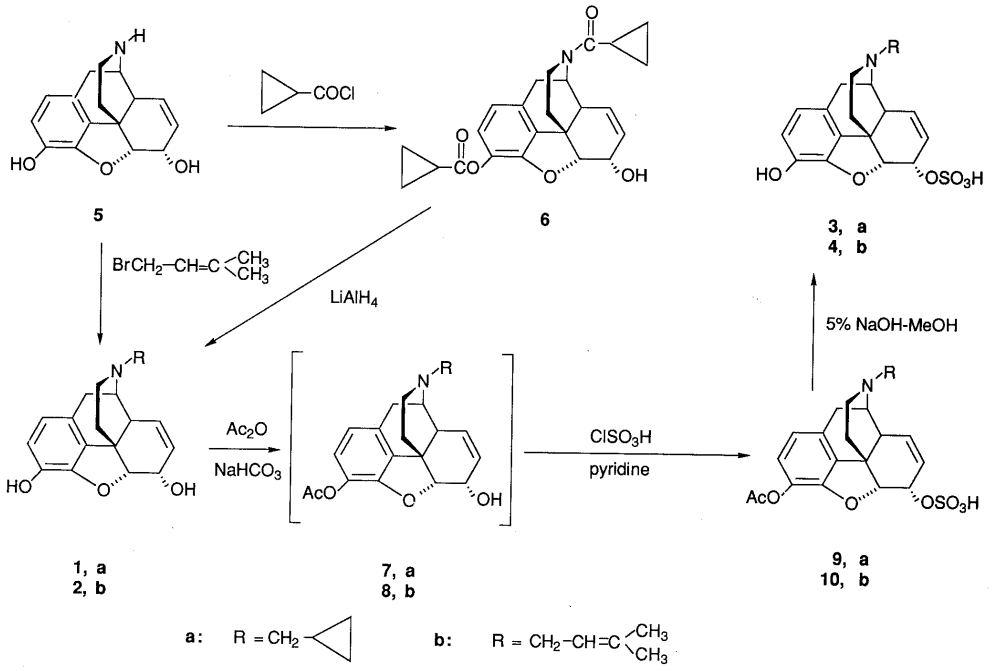
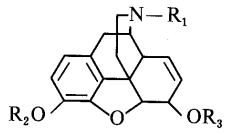


Chart 2

TABLE I. *N*-Substituted Normorphine Derivatives: Synthetic Yield and Melting Points



No.	R_1	R_2	R_3	Salt	Yield	mp (°C)	Recryst. solvent	Formula
1	Cyclopropylmethyl	H	H		74	189—191	MeOH	$C_{20}H_{23}NO_3 \cdot \frac{1}{2}CH_3OH \cdot \frac{1}{2}H_2O$
2	Dimethylallyl	H	H		49	97—99	Benzene	$C_{21}H_{25}NO_3 \cdot C_6H_6$
3	Cyclopropylmethyl	H	SO_3H		55	280—285[b]	MeOH	$C_{20}H_{23}NO_6S \cdot 1\frac{1}{2}H_2O$
4	Dimethylallyl	H	SO_3H		70	290—295[b]	MeOH	$C_{21}H_{25}NO_6S \cdot \frac{1}{2}H_2O$
5	H	H	H		63[a]	250—258[b]	MeOH	$C_{16}H_{17}NO_3 \cdot 1\frac{1}{2}H_2O$
6	Cyclopropylcarbonyl	Cyclopropylcarbonyl	H		100	176—178	AcOEt	$C_{24}H_{25}NO_5$
7	Cyclopropylmethyl	$COCH_3$	H		100	Oil		
8	Dimethylallyl	$COCH_3$	H		100	Oil		
9	Cyclopropylmethyl	$COCH_3$	SO_3H		70	245—247[b]	H_2O	$C_{22}H_{25}NO_7S \cdot 2\frac{1}{2}H_2O$
10	Dimethylallyl	$COCH_3$	SO_3H		70	248—250[b]	H_2O	$C_{23}H_{27}NO_7S \cdot H_2O$
11	Cyclopropylmethyl	H	H	HCl	100	191—193	Et_2O	$C_{20}H_{23}NO_3 \cdot \frac{1}{2}H_2O \cdot HCl$
12	Dimethylallyl	H	H	HCl	100	181—183	Et_2O	$C_{21}H_{25}NO_3 \cdot H_2O \cdot HCl$

a) Compound **5** was synthesized from morphine in about a 63% yield *via* three step reactions according to the method described in the literature.[4] *b*) Decomposition.

drugs at 9 a.m., 3 p.m. and 9 p.m. At 4 h after the last injection, the mice were challenged by a s.c. injection of nalorphine HCl at a dose of 10 mg/kg and physical dependence was assessed by using a jumping response as a criterion of precipitated withdrawal. Frequent jumping behavior was seen in mice treated chronically with CPN, C-6-S, N-6-S, morphine and morphine-6-sulfate (M-6-S), while, no such sign was observed by chronic treatment with DMN, D-6-S, nalorphine and pentazocine (Table IV). The development of physical dependence was, thus, strikingly enhanced by the sulfation of nalorphine and morphine at the 6-position, but, the dependence liability was not changed in pairs of *N*-cyclopropylmethyl and *N*-dimethylallyl derivatives by the modification. Tremors and diarrhea were also seen as the precipitated withdrawal signs in mice treated chronically with norphine, M-6-S and N-6-S.

Discussion

The present studies intended to examine the effect of modification on the pharmacological activity of *N*-allyl, *N*-cyclopropylmethyl and *N*-dimethylallyl derivatives of normorphine by sulfation at the 6-position. Newly synthesized C-6-S and D-6-S retained equipotent analgesic activity with that of the parent compounds, when assessed by the acetic acid writhing test with s.c. injection. The analgesic activity of N-6-S reported previously[9] was reconfirmed to be twice as potent as that of nalorphine also in the present study. On the other hand, the antagonistic activity of C-6-S and D-6-S to morphine analgesia was more potent than that of CPN and DMN, respectively. The sulfation appears to be more effective on the antagonistic effect than on the agonistic effect. The effect on the development of physical dependence by the modification

TABLE II. Analgesic Activity of Normorphine Derivatives Assessed by Acetic Acid Writhing Test in Mice

Drugs[a]	ED_{50}[b]	μmol/kg	Relative potency[c]
CPN	0.122	(0.097—0.151)	3.78
C-6-S	0.182	(0.137—0.242)	2.53
DMN	7.48	(5.10—11.9)	0.062
D-6-S	9.88	(6.98—14.0)	0.047
Nalorphine	1.43	(1.13—1.75)	0.322
N-6-S	0.716	(0.639—0.794)	0.644
Morphine	0.461	(0.384—0.547)	1.00

a) Drugs were injected subcutaneously. *b*) Values in parentheses represent 95% confidence limits. *c*) Values represent relative potency to that of morphine on the basis of ED_{50}.

TABLE III. Antagonistic Effect of Normorphine Derivatives and Pentazocine on Analgesic Activity of Morphine in Mice

Drugs	Dose nmol/body	Number of animals	Analgesia[a] (%)
Morphine	—	25	78.0
Morphine & CPN	0.1	10	30.0
	0.2	10	15.0
Morphine & C-6-S	0.01	10	30.0
	0.025	10	15.0
	0.05	10	5.0
	0.1	10	0
Morphine & DMN	25.0	20	55.0
	50.0	10	50.0
	100.0	10	40.0
Morphine & D-6-S	5.0	10	40.0
	10.0	10	30.0
	20.0	10	15.0
Morphine & nalorphine	0.8	10	35.0
Morphine & N-6-S	0.1	10	35.0
	0.2	10	20.0
Morphine & pentazocine	32.0	10	40.0

Morphine HCl (8 nmol/body) and antagonists were injected intracerebrally simultaneously. Analgesia was assessed 20 min after the injection by Haffner's tail pinch test. An ambiguous response to the test was counted as 0.5. *a*) Values represent % of mice which responded positively to the analgesic test.

TABLE IV. Naloxone-Precipitated Jumping in Mice after Chronic Administration of Normorphine Derivatives and Pentazocine

Drugs	Dose μmol/kg	Jumped mice/ total number	% of jumping response
CPN	100	4/15	26.7[a]
C-6-S	100	5/15	33.3[a,c]
DMN	200	0/15	0
D-6-S	200	0/7	0
Nalorphine	200	0/15	0
N-6-S	100	12/15	80.0[a,b]
Morphine	100	4/15	28.6[a]
	200	7/15	46.7[a]
M-6-S	10	9/13	69.2[a,d]
	50	12/13	92.3[a,e]
Pentazocine	200	0/14	0
Saline	—	0/42	0

Drugs were injected s.c. 14 times at 9 a.m., 3 p.m. and 9 p.m. of every day. Doses were raised gradually (1st and 2nd, 1/8; 3rd and 4th, 1/4; 5th and 6th, 1/2; 7th and 8th, 3/4; 9th to the last, the doses shown in this table). Naloxone HCl was challenged at a dose of 10 mg/kg 4 h after the last injection of test drugs, and a jumping response was observed for 15 min. In the drugs of low dependence liability, the doses were raised up to 200 μmol/kg. *a*) Significantly different from saline group, $p<0.01$ [χ^2-test]. *b*) Significantly different from nalorphine group, $p<0.01$ [χ^2-test]. *c*) Not significantly different from CPN group, $p>0.05$ [χ^2-test]. *d*) Significantly different from morphine group (100 μmol/kg), $p<0.05$ [χ^2-test]. *e*) Significantly different from morphine group (200 μmol/kg), $p<0.01$ [χ^2-test].

was not significant to *N*-cyclopropylmethyl and *N*-dimethylallyl derivatives in contrast to the striking effect on *N*-allyl and *N*-methyl derivatives.

The results obtained here extended previous findings on the pharmacological activity of morphine and nalorphine by the modification at the 6-position.[2,9] We first found very potent analgesic activity of morphine-6-glucuronide in mice.[1] Recently, the activity of this morphine metabolite was recognized to be 90- and 650-times as potent as that of morphine in mice when injected i.c.v. and intrathecally, respectively.[16] The sulfation of morphine at the same position also raised the analgesic activity above morphine similarly.[2] Further glucuronate and sulfate conjugates of nalorphine at the 6-position showed more potent antagonistic activity to morphine analgesia.[9] These findings seemed to suggest that the glucuronidation and sulfation of morphine and related compounds at the 6-position raise either agonistic or antagonistic activity directed to the opioid receptors.

In the present study, the analgesic activity of the sulfates of *N*-cyclopropylmethyl and *N*-dimethylallyl derivatives was equipotent to that of the parent compounds by the s.c. route of administration. This also agreed with the previous finding that nalorphine-6-conjugates slightly raised the analgesic effect of nalorphine.[9] This result means that the sulfated antagonists do not lose, but do not raise significantly the analgesic effect when injected s.c.

There was inconsistency in the effect of sulfation on the development of physical dependence. Remarkable withdrawal signs were reconfirmed in mice treated chronically with N-6-S by challenge with naloxone, while no such signs were observed in the case of nalorphine.[9] This effect of nalorphine-6-sulfate made a striking contrast to the indistinguishable activity of CPN and C-6-S, and of DMN and D-6-S. DMN is a weak analgesic and this may be the reason that D-6-S did not produce addiction, the dependence liability of a strong agonist CPN by sulfation was also not changed. Although the reason for this inconsistency has not yet been resolved, it seems to be due to the different affinity and selectivity of the above compounds to the opioid receptors. Gilbert and Martin have indicated that nalorphine is an antagonist at the μ-receptor and a partial agonist at the κ-receptor.[17] They also suggested that each of the κ-receptor-directed mixed agonist–antagonists had a different affinity and selectivity to the κ-receptor, and that cyclazocine possessing a cyclopropylmethyl group like CPN was a strong agonist to the κ-receptor. Therefore, the different effect of the 6-conjugates on the development of physical dependence seems to be induced by the altered interactions with the multiple opioid receptors.[18]

Experimental

1) Chemistry Melting points were determined on a Yanagimoto melting point microscope and were uncorrected. Infrared (IR) spectra were obtained on a JEOL DS-701G spectrometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined with a JEOL DS-100 spectrometer. Chemical shifts are expressed in ppm (δ) relative to tetramethylsilane as an internal standard and $CDCl_3$ or dimethyl sulfoxide-d_6 (DMSO-d_6) was used as a solvent. Mass spectra (MS) were obtained on a JEOL D-300 spectrometer.

Reaction progress and purity of products were determined by analytical thin layer chromatography (TLC) using precoated plates (either Merck silica gel G or Wako B-5FM, 20 × 5 cm or 20 × 20 cm). Spots were visualized

with a ultraviolet (UV) 254 nm light or iodine vapor. Silica gel type 60 (Merck, 70—230 mesh) and Silicic AR CC-7 (Mallinckrodt) were used for column chromatography. Elemental analyses were performed at the Analytical Section, Kyushu University. Organic extracts were dried over anhydrous Na_2SO_4.

Normorphine (5) Compound **5** was synthesized from morphine according to the method described in the literature[14] in about a 63% yield *via* diacetylmorphine (92% yield) and *N*-cyanodiacetylnormorphine (80% yield) as a light brown powder. UV λ_{max}^{EtOH} nm (log ε): 282 (3.25). MS *m/z*: 271 (M^+). *Anal.* Calcd for $C_{16}H_{17}NO_3 \cdot 1\ 1/2\ H_2O$: C, 64.40; H, 6.76; N, 4.69. Found: C, 64.45; H, 6.66; N, 4.68.

3-*O*,*N*-Dicyclopropylcarbonylnormorphine (6) A solution of cyclopropylcarbonyl chloride (3.74 g, 35.8 mmol) in $CHCl_3$ (11 ml) was added dropwise to a stirring mixture of **5** (2.15 g, 7.2 mmol), triethylamine (6.5 ml), and $CHCl_3$ (43 ml) during a period of 5 min. The mixture was refluxed for 8 h. After being cooled, the mixture was washed twice with 60 ml of 3.6% HCl, and then washed twice with 60 ml of 5% Na_2CO_3. The $CHCl_3$ layer was dried and concentrated *in vacuo*. The oily residue was crystallized from ethyl acetate to give **6** (almost a quantitative yield) as colorless needles. IR ν_{max}^{KBr} cm^{-1}: 1741 (C=O); 1631 (C=O). UV λ_{max}^{EtOH} nm (log ε): 287 (3.17). ^{1}H-NMR ($CDCl_3$) δ: 4.12—4.32 (1H, m, C_6-H), 4.93 (1H, d, J=7 Hz, C_5-H), 5.20—5.40 (1H, m, C_8-H), 5.68—5.96 (1H, m, C_7-H), 6.70 (2H, dd, J=8 Hz, C_1-H, C_2-H). MS *m/z*: 407 (M^+). *Anal.* Calcd for $C_{24}H_{25}NO_5$: C, 70.74; H, 6.18; N, 3.44. Found: C, 70.45; H, 6.16; N, 3.38.

***N*-Cyclopropylmethylnormorphine (1)** To a stirred solution of **6** (2.84 g, 7.0 mmol) in tetrahyrofuran (THF) (70 ml) was added dropwise a suspension of lithium aluminum hydride (1.26 g, 33.2 mmol) in THF (10 ml) at room temperature during a period of 5 min. The resulting mixture was stirred at room temperature for an additional 23 h. The remaining lithium aluminum hydride was allowed to decompose by the cautious addition of ethyl acetate (4 ml) and H_2O (6 ml). After standing, the upper layer was removed, and the lower layer was extracted three times with 75 ml of ethyl acetate. The combined extract was dried and concentrated *in vacuo*. The residue was recrystallized from MeOH to give **1** (1.84 g, 74% yield) as light brown crystals. UV λ_{max}^{EtOH} nm (log ε): 287 (3.19). ^{1}H-NMR ($CDCl_3$) δ: 0—1.10 (5H, m, cyclopropane-H), 2.49 (2H, d, J=6 Hz, *N*-C<u>H</u>$_2$-cyclopropyl), 3.45 (1.5H, s, C<u>H</u>$_3$OH), 4.07—4.27 (1H, d, C_6-H), 4.83 (1H, d, J=6 Hz, C_5-H), 5.12—5.36 (1H, m, C_8-H), 5.53—5.76 (1H, m, C_7-H), 6.53 (2H, dd, C_1-H, C_2-H). MS *m/z*: 325 (M^+). *Anal.* Calcd for $C_{20}H_{23}NO_3 \cdot 1/2H_2O \cdot 1/2CH_3OH$: C, 70.36; H, 7.34; N, 3.34. Found: C, 70.48; H, 7.64; N, 3.79.

***N*-Dimethylallylnormorphine (2)** Dimethylallyl bromide (0.82 g, mmol) was added slowly to a stirred mixture of **5** (1.58 g, 5.3 mmol) and K_2CO_3 (2.5 g, 18.1 mmol) in dimethylformamide (DMF) (25 ml). The resulting mixture was heated at 90—95 °C for 3 h under N_2. After being cooled, the reaction mixture was diluted with H_2O (100 ml), saturated with NaCl, and extracted with three 30 ml parts of $CHCl_3$. The combined extracts were washed with a saturated NaCl solution (100 ml), dried, and concentrated *in vacuo*. The brown oily residue was chromatographed on silica gel ($CHCl_3$ and 2% MeOH/$CHCl_3$) to give **2** (1.50 g, 83% yield) as an oil. It was crystallized from benzene to give **2** (1.06 g, 49% yield) as light brown crystals. UV λ_{max}^{EtOH} nm (log ε): 286.5 (3.16). ^{1}H-NMR ($CDCl_3$) δ: 1.68 and 1.72 (3H×2, s, *gem* CH_3), 3.14 (2H, d, *N*-C<u>H</u>$_2$-CH=), 4.05—4.25 (1H, m, C_6-H), 4.82 (1H, d, C_5-H), 5.12—5.40 (1H×2, m, –CH_2–C<u>H</u>=C and C_8-H), 5.50—5.74 (1H, m, C_7-H), 6.53 (2H, dd, C_1-H), C_2-H), 7.34 (6H, s, benzene-H). *Anal.* Calcd for $C_{21}H_{25}NO_3 \cdot C_6H_6$: C, 77.67; H, 7.48; N, 3.35. Found: C, 77.56; H, 7.53; N, 3.28. MS *m/z*: 339 (M^+).

***N*-Cyclopropylmethylnormorphine·HCl (11)** HCl gas was bubbled into a solution of **1** (436 mg, 1.2 mmol) in dry ether (30 ml). The resulting precipitate was collected by filtration, washed with dry ether, and dried *in vacuo* to give **11** (almost quantitative yield) as colorless crystals. UV λ_{max}^{EtOH} nm (log ε): 285 (3.14). *Anal.* Calcd for $C_{20}H_{23}NO_3 \cdot 1/2H_2O \cdot HCl$: C, 64.77; H, 6.79; N, 3.78. Found: C, 65.13; H, 7.17; N, 3.52.

***N*-Dimethylallylnormorphine·HCl (12)** This compound was prepared from **2** in an almost quantitative yield in the same manner as compound **11**. UV λ_{max}^{EtOH} nm (log ε): 285 (3.16). *Anal.* Calcd for $C_{21}H_{25}NO_3 \cdot H_2O \cdot HCl$: C, 64.03; H, 7.16; N, 3.56. Found; C, 64.02; H, 7.25; N, 3.35.

3-*O*-Acetyl-*N*-cyclopropylmethylnormorphine (7) Acetic anhydride (2.7 ml) was added dropwise to a stirring suspension of **1** (501.9 mg, 1.4 mmol) and $NaHCO_3$ (5 g, 59.5 mmol) in H_2O (100 ml) during a period of 10 min. The resulting mixture was stirred at room temperature for 30 min. The reaction mixture was then extracted three times with 100 ml of $CHCl_3$. The combined extracts were washed with H_2O (200 ml), dried and concentrated *in vacuo* to give **7** (an almost quantitative yield) as an oily product. The product showed a spot (*Rf* 4.8) by silica gel TLC with a solvent system of $CHCl_3$ and MeOH (9 : 1) and detection with potassium platinum iodide reagent. Compound **7** was used without further purification.

3-*O*-Acetyl-*N*-dimethylallylnormorphine (8) This compound was prepared from **2** in an almost quantitative yield in the same manner as compound **7**. The product gave a spot (*Rf* 4.5) by silica gel TLC with a solvent system of $CHCl_3$ and MeOH (9 : 1) and detection with potassium platinum iodide reagent.

3-*O*-Acetyl-*N*-cyclopropylmethylnormorphine-6-*O*-sulfate (9) A mixture of dry pyridine (1.2 ml) and chlorosulfonic acid (0.2 ml) was heated at 60 °C for 10 min. To this, a solution of **7** (593.4 mg, 1.6 mmol) in dry pyridine (0.4 ml) was added and heated at 70 °C for 10 min. To jelly clump separated as a lower layer was added 5 ml of H_2O. After standing for a while, the solution was concentrated *in vacuo* to remove pyridine. H_2O (5 ml) was added to the oily residue, and the resultant precipitate was collected by filtration, recrystallized from H_2O to give **9** (504.7 mg, 70%) as colorless crystals. IR ν_{max}^{KBr} cm^{-1}: 1019 (SO_2), 1240 (SO_2), 1750 (C=O), 2720 $(NH)^+$. UV λ_{max}^{EtOH} nm (log ε): 285 (3.18). *Anal.* Calcd for $C_{22}H_{25}NO_7S \cdot 2\ 1/2H_2O$: C, 53.65; H, 6.14; N, 2.83. Found: C, 53.49; H, 5.99; N, 2.98.

3-*O*-Acetyl-*N*-dimethylallylnormorphine-6-*O*-sulfate (10) This compound was prepared from **8** in a 70% yield in the same manner as compound **9**. IR ν_{max}^{KBr} cm^{-1}: 1022 (SO_2), 1250 (SO_2), 1756 (C=O), 2755 $(NH)^+$. UV λ_{max}^{EtOH} nm (log ε): 281.5 (3.30). *Anal.* Calcd for $C_{23}H_{28}NO_7S \cdot H_2O$: C, 57.61; H, 6.10; N, 2.92. Found: C, 57.38; H, 6.12; N, 3.09.

***N*-Cyclopropylmethylnormorphine-6-*O*-sulfate (3)** A solution of **9** (491.5 mg, 1.0 mmol) in 5% NaOH–MeOH (25 ml) was stirred at room temperature for 1 h. After being adjusted to pH 6.5 with 10% acetic acid, the reaction mixture was concentrated *in vacuo*. The oily residue was crystallized from MeOH to give **3** (223.5 mg, 55%) as colorless crystals. IR ν_{max}^{KBr} cm^{-1}: 1017 (SO_2), 1260 (SO_2), 2720 $(NH)^+$. UV λ_{max}^{EtOH} nm (log ε): 282 (3.31). *Anal.* Calcd for $C_{20}H_{23}NO_6S \cdot 1\ 1/2H_2O$: C, 57.68; H, 6.29; N, 3.36. Found: C, 57.35; H, 5.93; N, 3.34.

***N*-Dimethylallylnormorphine-6-*O*-sulfate (4)** This compound was prepared from **10** in a 70% yield in the same manner as compound **3**. IR ν_{max}^{KBr} cm^{-1}: 1018 (SO_2), 1249 (SO_2), 2760 (NH^+). UV λ_{max}^{EtOH} nm (log ε): 285.5 (3.18). *Anal.* Calcd for $C_{21}H_{25}NO_6S \cdot 1/2H_2O$: C, 58.86; H, 6.12; N, 3.27. Found: C, 59.26; H, 6.15; N, 3.12.

2) Pharmacology Materials Male mice (ddN strain) weighing 15—20 g were used for pharmacological experiments. Nalorphine hydrochloride was supplied from the Ministry of Health and Welfare of Japan. Morphine hydrochloride was purchased from Takeda Chemical Ind., Ltd. (Osaka). Pentazocine and naloxone hydrochloride were kindly given by Sankyo Co., Ltd. (Tokyo). M-6-S and N-6-S were synthesized according to the method reported previously.[2,9] CPN and DMN were used as HCl salt (**11** and **12**), respectively.

Analgesic Activity Analgesic activity was assessed by the acetic acid writhing method developed by Koster *et al.*[19] Writhings were counted for 10 min from 10 min after an i.p. injection of 0.6% acetic acid physiological saline solution. Drugs were dissolved in the saline and injected s.c. 30 min before the injection of acetic acid at a volume of 0.1 ml/10 g body weight. The time to estimate the analgesia was decided following the maximum effect of morphine HCl and morphine-6-glucuronide at about 45 min after the injection s.c.[1]

Antagonistic Activity Antagonistic activity to morphine analgesia was assessed by the tail-pinch method in mice.[20] Drugs were dissolved in physiological saline and injected i.c.v. concomitantly with 8 nmol morphine HCl at a volume of 20 μl/body. Time for determination of the activity was set at 20 min after the injection from the result of a preliminary study for the time point of the most reproducible and potent effect of morphine and test drugs.

Development of Physical Dependence Dependence was induced in 7—42 mice by giving drugs or saline s.c. 14 times totally at 9 a.m., 3 p.m. and 9 p.m. everyday. Dosage was increased gradually (1st-2nd, 1/8; 3th—4th, 1/4; 5th—6th, 1/2; 7th—8th, 3/4; 9th—14th, the same dose of the last injection). The mice were challenged with 10 mg/kg s.c. injection of naloxone HCl 4 h after the last injection. The jumping response and other withdrawal signs (tremor, diarrhea) were observed for 15 min after the injection of naloxone following the method of Way *et al.*[21]

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