Synthesis and Analgesic Effect of Normorphine-3- and -6-glucuronides

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Normorphine-3- and -6-glucuronides were synthesized, and their analgesic effects were examined. Normorphine-3-glucuronide was obtained by condensation of normorphine with acetobromoglucuronate in the presence of sodium hydroxide in acetone. On the other hand, normorphine-6-glucuronide was synthesized by condensing N,O^3-biscarbobenzoxynormorphine with acetobromoglucuronate in the presence of silver carbonate, and removing the protecting groups from the resultant reaction product by catalytic hydrogenation and solvolysis with sodium methoxide and barium hydroxide. The analgesic effect of normorphine-6-glucuronide (ED_{50} 0.036 nmol/mice) was 125-fold more potent than that of normorphine in mice injected i.c.v. Normorphine-3-glucuronide was shown to be 37% effective at a dose of 2 nmol/mice, but induced convulsion at higher doses when given by i.c.v injection.

Keywords normorphine; morphine; narcotics; analgesics; glucuronide; metabolite; chemical synthesis; normorphine glucuronide; analgesic effect; mouse

Morphine metabolism has been extensively studied and mainly involves glucuronidation, *N*-demethylation, sulfation, and dehydrogenation.[1-3] The major metabolic pathway is glucuronidation, which yields two glucuronate isomers; an inactive major metabolite, morphine-3-glucuronide (M-3-G), and a minor metabolite, morphine-6-glucuronide (M-6-G), possessing a potent analgesic effect.[4] A recent clinical study suggested that most of the analgesic effect of morphine is due to M-6-G in cancer patients.[5,6]

Another important pathway in morphine metabolism is formation of normorphine by oxidative *N*-demethylation. Elliott *et al.* reported expiration of [^{14}C]carbon dioxide in volunteers administered [*N*-^{14}C-methyl]morphine.[7] [^{14}C]-Carbon dioxide was also recovered in breath of rats[8] and dogs[9] injected with [*N*-^{14}C-methyl]morphine. Further, numerous studies have suggested possible excretion of conjugated normorphines as urinary metabolites of morphine.[10-16] In 1977, Yeh *et al.* detected normorphine-6-glucuronide (NM-6-G) along with unchanged morphine, free normorphine, M-3-G, M-6-G, morphine-3,6-diglucuronide and morphine-3-sulfate in the urine of volunteers administered morphine.[17] They also suggested excretion of normorphine-3-glucuronide (NM-3-G) as a possible metabolite. NM-3-G was isolated as the major metabolite in dogs administered normorphine, although the presence of NM-6-G was not referred to.[18] Chemical synthesis of normorphine glucuronides, however, has not yet been reported and would be useful to stimulate further studies on their metabolic and pharmacological roles in morphine treatment. We now wish to report the synthesis and analgesic effect of NM-3-G and NM-6-G.

Recently, numerous methods for chemical synthesis of glucuronate conjugates were reviewed by Kaspersen and Van Boeckel.[19] Normorphine has three available groups for glucuronidation. Selective glucuronidation of the phenolic hydroxyl group, in the present study, was performed as shown in Chart 1, following the same method used for synthesis of M-3-G[20] and nalorphine-3-glucuronide.[21] The starting material, normorphine, was obtained from morphine according to the method of Rapoport and Look.[22] The slow rate of the condensation reaction caused solvolysis of acetobromoglucuronate with alkali and precipitation of unreacted normorphine. To complete the reaction, therefore, repeated additions of alkali and the glucuronate were required. The reaction directly proceeded to the final product **4** due to alkaline hydrolysis of the intermediate **3**. Compound **4**, colorless fine needles, mp 252—257 °C (dec.) was obtained by purification with a Dowex 50W-X8 (H^+-form) column.

Compound **4** showed a ultraviolet (UV) absorption spectrum analogous to that of normorphine, and exhibited no bathochromic shift in alkaline solution. The infrared (IR) absorption spectrum indicated a marked absorption peak at 1597 cm^{-1}, due to carboxylic ion, suggesting a zwitterionic structure of this compound. Treatment with β-glucuronidase liberated normorphine. Based on these data, including elemental analysis, we concluded that compound **4** is normorphin-3-yl-β-D-glucopyranosiduronate.

Synthesis of NM-6-G was conducted as shown in Chart 2. Prior to condensation of normorphine with the glucuronate at the 6-position, reactive groups in normorphine except for the alcoholic group were protected with carbo-

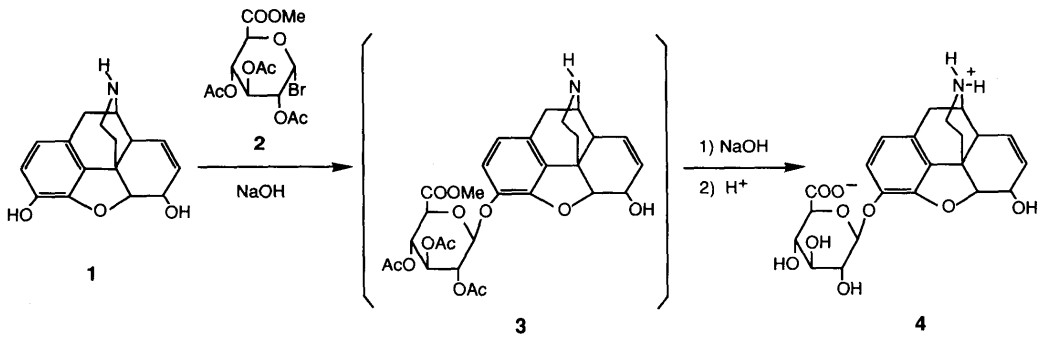

Chart 1

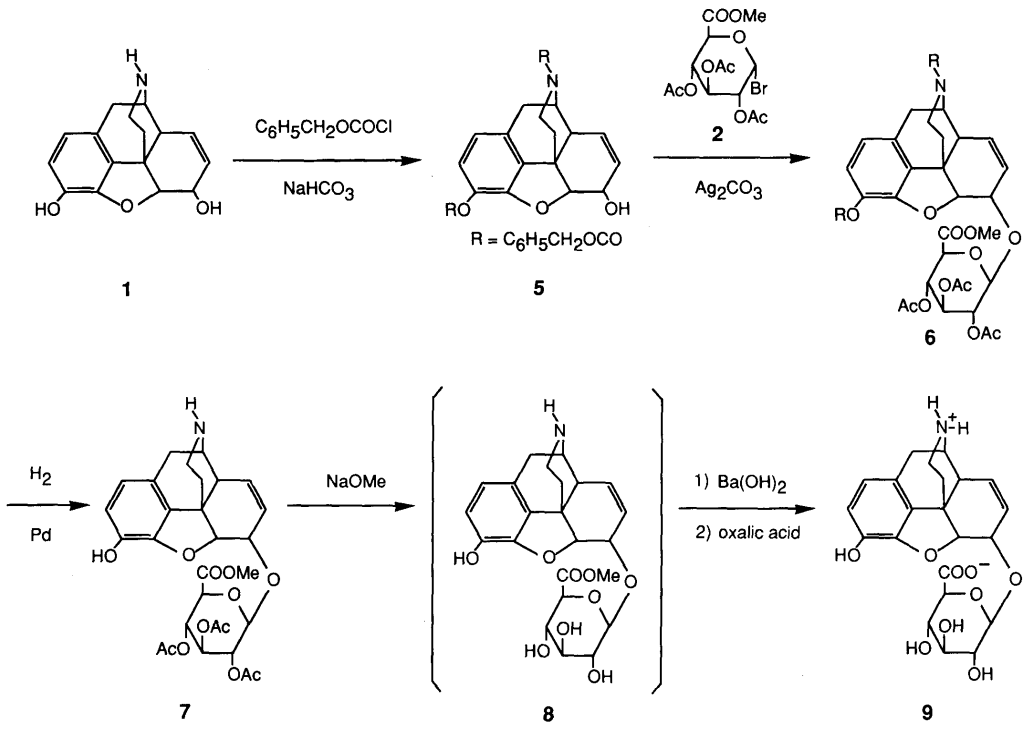

Chart 2

TABLE I. Analgesic Effect of NM-3-G and NM-6-G in Mice

Drug	ED_{50} (nmol/mouse)	Relative potency
Morphine[a]	1.9 (1.2—3.0)	2.4
Normorphine	4.5 (3.1—6.6)	1.0
NM-3-G	>2.0[b]	—
NM-6-G	0.036 (0.017—0.078)	125

Male ddY mice were given 10 μl of physiological saline solution of drugs by i.c.v. injection, and the analgesic effect was assessed by means of the tail-pinch test[23] at 15 min after the injection. Values in parentheses show 95% confidence limits. *a*) Data were taken from reference 4. *b*) NM-3-G was positive in 3 mice out of 8 at the dose of 2 nmol/mice, but higher doses caused convulsion.

benzoxy chloride. The resultant derivative of normorphine was then subjected to the classical Koenig–Knorr reaction, as used for the synthesis of M-6-G[20] and nalorphine-6-glucuronide.[21] Compound **6** was obtained as an oily substance. The carbobenzoxy groups were removed by catalytic hydrogenation with palladium on activated charcoal. Acetyl and methyl groups in the glucuronic acid moiety were removed with sodium methoxide and barium hydroxide, respectively. The final product, compound **9**, was obtained as pale brown needles, mp 290—292 °C (dec.). The UV absorpion spectrum indicated the presence of a phenolic hydroxyl group (bathochromic shift to 298 nm in alkali from 285 nm in water). The IR absorption spectrum supported a zwitterionic form (carboxylic ion absorption at 1597 cm^{-1}). The product liberated normorphine upon hydrolysis with β-glucuronidase. These results, together with elemental analysis, indicated that compound **9** is normorphin-6-yl-β-D-glucopyranosiduronate.

The analgesic effect of NM-3-G and NM-6-G was assessed by means of the tail-pinch test[24] in mice at 15 min after i.c.v. injection. ED_{50} is shown in Table I. NM-6-G demonstrated 125-fold more potent analgesic effect than that of normorphine. NM-3-G was effective in 3 mice out of 8 at a dose of 2 nmol/mice, but higher doses of this glucuronide caused convulsion. The present result provides further evidence of an enhanced analgesic effect as a consequence of glucuronidation and sulfation of the 6-position of morphine.[4,25] Recently, the potential significance of M-6-G in the clinical effect of morphine was suggested.[6] NM-6-G has been detected as a urinary metabolite of morphine in man,[18] but the plasma level of this glucuronide in man needs to be determined before the significance of NM-6-G in morphine analgesia can be evlauated.

Experimental

Melting points were determined on a hot stage, and are uncorrected. UV absorption spectra were obtained with a Hitachi double-beam spectrometer, model 340. IR absorption spectra were taken on a JASCO IR spectrometer, model DS-701G. Morphine hydrochloride was purchased from Takeda Chem. Ind. Co., Ltd., Osaka. Normorphine was synthesized from morphine according to the method of Rapoport and Look.[22] β-Glucuronidase (*Escherichia coli* type IX) was purchased from Sigma Chem. Co., St. Louis, Mo., U.S.A.

Synthesis of Normorphin-3-yl-glucopyranosiduronate (4) (NM-3-G) Acetobromoglucuronate (600 mg) dissolved in acetone (2.0 ml) was added dropwise to normorphine (300 mg) dissolved in 3.0 N NaOH (1.0 ml), and the mixture was allowed to stand in the dark. Unreacted normorphine precipitate was dissolved again by addition of 30% sodium hydroxide (0.5 ml), and acetobromoglucuronate (150 mg) dissolved in acetone (0.5 ml) was added. In total, 5 ml of 30% sodium hydroxide and 1.5 g of the glucuronate were added to this mixture. The reaction mixture was diluted to 20 ml with water, and extracted with a mixture of $CHCl_3$ and MeOH (3 : 1, by vol.). The extract was evaporated *in vacuo*. The residue was dissolved in water (100 ml) and poured onto an Amberlite XAD-2 column (100 ml). The column was washed with water (200 ml) and the product was eluted with MeOH (300 ml). A part of NM-3-G crystallized, and was collected by filtration. The filtrate was applied to a Dowex 50W-X8 (H^+-form) column (30 ml) in a cold room (4 °C). The column was washed with water (75 ml) and the product was eluted with 0.5 N aqueous NH_3 (150 ml). The UV positive fractions of filter paper were combined and concentrated *in vacuo*. NM-3-G was recrystallized from water as colorless fine needles. mp 252—254 °C (dec.). Yield 41 mg (8%). *Anal.* Calcd for $C_{22}H_{25}NO_9 \cdot$

$3.5H_2O$: C, 51.76; H, 6.31; N, 2.74. Found: C, 51.30; H, 6.25; N, 2.67. IR (KBr): 1597 (C=O) cm^{-1}. UV $\lambda_{max}^{H_2O}$ nm (log ε): 282.5 (3.12).

Synthesis of N,O^3-Biscarbobenzoxynormorphine (6) Carbobenzoxy chloride (0.23 ml) was added to a suspension of normorphine (420 mg) in 5% sodium bicarbonate solution (4.5 ml). The reaction was continued to give a single spot on silica gel thin layer chromatography (TLC) with $CHCl_3$–MeOH (9 : 1, by vol.). The product was extracted with $CHCl_3$ and dried on anhydrous Na_2SO_4. Unreacted carbobenzoxy chloride was removed by evaporation *in vacuo* at 95 °C. Yield 790 mg (oil). MS *m/z*: 539 (M^+).

Synthesis of Normorphin-6-yl-β-D-glucopyranosiduronate (9) (NM-6-G) Silver carbonate (930 mg) and acetobromoglucuronate (960 mg) were added in 5 portions to N,O^3-biscarbobenzoxynormorphine (770 mg) dissolved in benzene (5.0 ml), and the mixture was refluxed for approximately 30 h. The filtrate was evaporated *in vacuo*. The residue was dissolved in $CHCl_3$ (4.0 ml), then MeOH (10 ml) and Pd–C (50 mg) were added, and the mixture was stirred under an H_2 stream until no further $BaCO_3$ precipitate appeared. The filtrate was evaporated *in vacuo*. The residue was dissolved in 0.43 N $Ba(OH)_2$, allowed to stand for 2 h, and adjusted to pH 6.0 with 2.0 N oxalic acid. The filtrate was adjusted to pH 9 with aqueous NH_3, extracted with $CHCl_3$–iso-PrOH (3 : 1, by vol.), and evaporated *in vacuo*. The residue was dissolved in water (10 ml) and poured onto an Amberlite XAD-2 column (10 ml). The column was washed with water (10 ml) and the product was eluted with MeOH (30 ml). The eluate was evaporated *in vacuo*. NM-6-G was recrystallized from water to pale brown needles. mp 290—292 °C (dec.). Yield 4 mg (0.6%). *Anal.* Calcd for $C_{22}H_{25}NO_9 \cdot 2H_2O$: C, 54.65; H, 6.04; N, 2.90. Found: C, 54.74; H, 6.23; N, 2.86. IR (KBr): 1597 (C=O) cm^{-1}. UV $\lambda_{max}^{0.1\,N\,NaOH}$ nm (log ε): 298 (3.44).

Measurement of Analgesic Effect Male ddY mice weighing 18—22 g were used in this experiment. Drugs dissolved in physiological saline (10 μl) were injected i.c.v. Analgesic effect was assessed by the tail-pinch method.[23] ED_{50} and the 95% confidence limit were calculated by the Litchfield–Wilcoxon method.[24]

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