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Metabolism of Drugs. LX.[1] The Synthesis of Codeine and Morphine Glucuronides[2]

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The first synthesis of three glucuronides of narcotics was reported. Codeine glucuronide (Va) was prepared by the condensation of codeine (Ia) with acetobromo sugar derivative (II) in the presence of silver carbonate and the following removal of the protecting groups by solvolysis and hydrolysis with sodium methoxide and aq. barium hydroxide, respectively. Morphine-6-glucuronide (Vb) was synthesized similarly to Va utilizing 3-acetylmorphine (Ib) as the starting material. Morphine-3-glucuronide (VIII) was prepared by the condensation of morphine (VI) with II in sodium hydroxide–acetone. In this reaction, the intermediate derivative (VII) was not obtained but hydrolyzed to the free glucuronide (VIII).

It has been known that conjugation is a major pathway for the detoxication of codeine and morphine. Thus, Woods,[4] and Fujimoto and Way[5] isolated the conjugated morphine in a crystalline form from the dog bile and from the human urine, after dosing morphine, respectively and characterized it as morphine-3-glucuronide by the elemental analysis and spectral measurement. The presence of other conjugated metabolites has been also suggested,[4,6] but no experimental evidences have been presented so far. Major metabolites of codeine were also suggested to be glucuronides,[7] but the conclusive evidence was not obtained. The chemical synthesis of these conjugates may consequently be of use for the identification of urinary metabolites and also for the pharmacological and toxicological studies. The present paper describes the first synthesis of such glucuronides as codeine glucuronide (Va), morphine-6-glucuronide (Vb) and morphine-3-glucuronide (VIII).

One of the limited informations concerning the synthesis of glycosides of narcotics is an early report of Mannich[8] who prepared the phenolic glucoside of morphine by the condensation of morphine with acetobromo derivative of glucose in a medium of sodium hydroxide–acetone and ether. Later, the same method was extended to the synthesis of a series of morphine-3-glycosides by Casparis and Béchert.[9] Casparis and his coworkers also prepared a series of codeine glycosides utilizing Koenigs–Knorr method.[10] These methods seemed effective also for the present investigation.

Codeine glucuronide (Va) was thus synthesized without any difficulty by the utilization of Koenigs–Knorr reaction as shown in Chart 1.

1) Part LIX: H. Yoshimura, H. Shimeno and H. Tsukamoto, *Biochem. Pharmacol.*, **17**, 1511 (1968).
2) This study was shortly communicated in *Tetrahedron Letters*, **1968**, 483.
3) Location: *Katakasu, Fukuoka.*
4) L.A. Woods, *J. Pharmacol. Exptl. Therap.*, **112**, 158 (1954).
5) J.M. Fujimoto and E.L. Way, *J. Pharmacol. Exptl. Therap.*, **121**, 340 (1957); *idem*, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 273 (1958).
6) V. Thompson and E.G. Gross, *J. Pharmacol. Exptl. Therap.*, **72**, 138 (1941).
7) J. Axelrod and J.K. Inscoe, *Proc. Soc. Exp. Biol. Med.*, **103**, 675 (1960).
8) C. Mannich, *Ann.*, **394**, 223 (1912).
9) P. Casparis and P. Béchert, *Pharm. Acta Helv.*, **22**, 97 (1947).
10) P. Casparis, E. Kühni and E. Leinzinger, *Pharm. Acta Helv.*, **24**, 145 (1949).

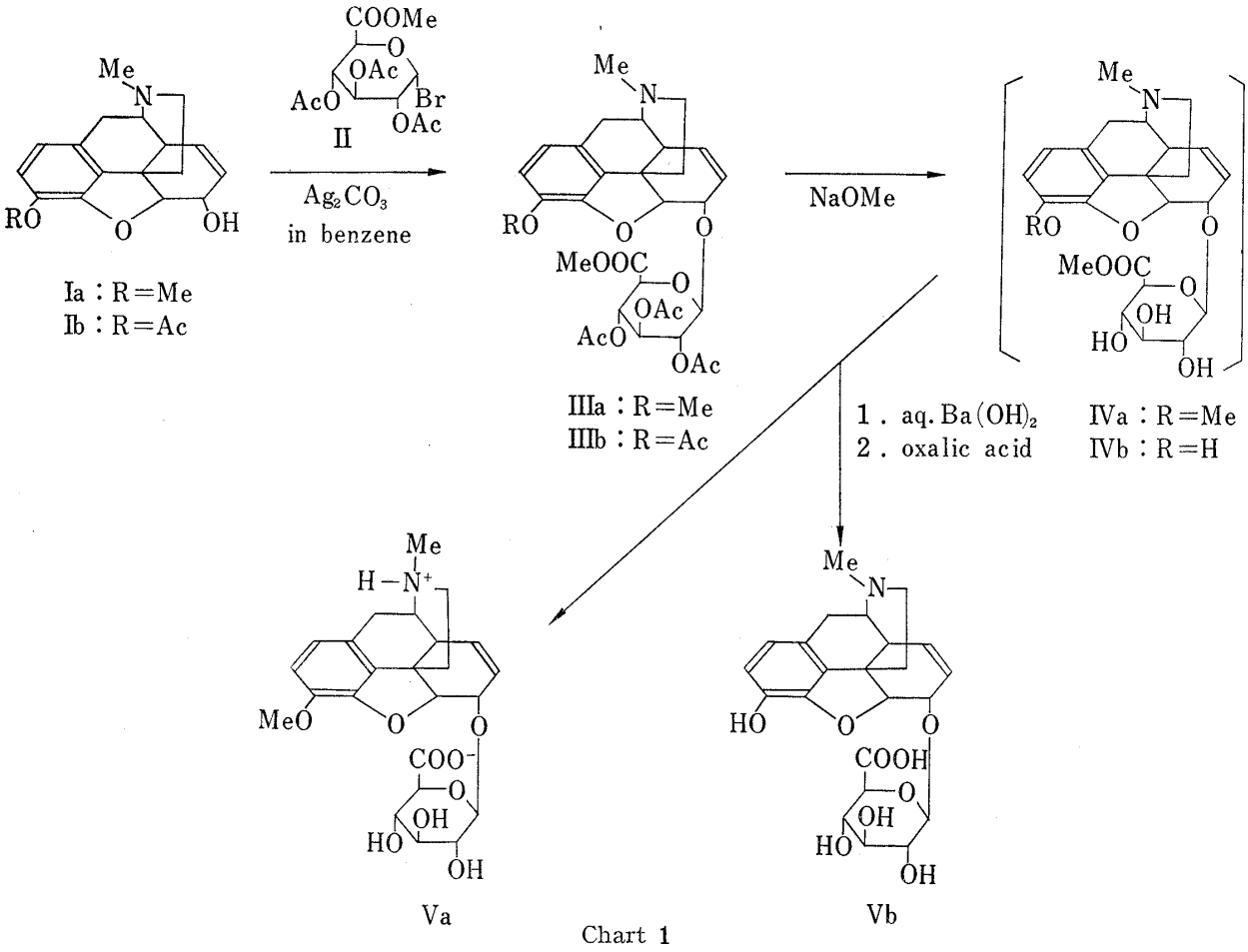

Chart 1

The first step of this synthetic route, the condensation of codeine (Ia) and acetobromo derivative of glucuronic acid (II) was performed in benzene, in the presence of silver carbonate. The reaction rate was considerably slow and so a prolonged heating (about 10 hours) was required to accomplish the reaction as compared with the ordinary case.[11] The reaction process could be checked conveniently by thin-layer chromatography which is shown in Fig. 1.

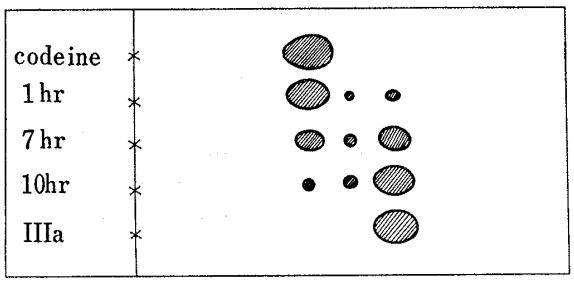

Fig. 1. Thin-Layer Chromatogram of Koenigs-Knorr Reaction Mixture
solvent system: $CHCl_3$–MeOH (4:1)
color reagent: potassium platinum iodide reagent

Rapoport and Reist reported previously that codeine was oxidized to codeinone with a large amount of silver carbonate in boiling benzene,[12] but this did not take place on slightly modified condition in the present study. Spectral data (Table I) of the reaction product were in a good accordance with the expected structure of the methyl acetyl derivative (IIIa). β-Configuration of the glycosidic linkage was supported by the optical rotation, and further confirmed indirectly by nuclear magnetic resonance (NMR) spectrum (60 Mc, $CDCl_3$). The axial C_1–H signal around 5.75 ppm ($J_{1,2}$=6—8 cps)[13] which should be present in the β-isomer was not clear due to overlapping with that of C_7–H signal (5.71 ppm, J=9.6 cps) of codeine,[14] while any

11) H.H. Wotiz, E. Smakula, N.N. Lichtin and J.H. Leftin, *J. Am. Chem. Soc.*, **81**, 1704 (1959).
12) H. Rapoport and H.H. Reist, *J. Am. Chem. Soc.*, **77**, 490 (1950).
13) L.D. Hall, *Tetrahedron Letters*, **1964**, 1457.
14) S. Okuda, S. Yamaguchi, Y. Kawazoe and K. Tsuda, *Chem. Pharm. Bull.* (Tokyo), **12**, 104 (1964).

signal was not observed around 6.31 ppm ($J_{1,2}$=3 cps), which could be seen as the equatorial C_1–H if it is α-isomer.

Removal of the protecting groups of IIIa was performed stepwise according to the method of Helferich and Berger;[15] IIIa was first converted to IVa on solvolysis with a catalytic amount of sodium methoxide and then to Va on hydrolysis with an equivalent amount of aqueous barium hydroxide and the following treatment with oxalic acid.

Infrared (IR) spectrum of Va showed a strong peak at 1609 cm^{-1} and a broad absorption between 2800 and 2400 cm^{-1}, suggesting the existence of an ionized carboxyl group and a positive charge on the piperidine nitrogen, respectively, as shown in Chart 1.

The synthesis of morphine-6-glucuronide (Vb) was similar to that of codeine glucuronide (Va) utilizing 3-acetylmorphine (Ib) as the starting material which was prepared quantitatively by selective acetylation of morphine according to the method of Welsh.[16] The NMR spectrum of IIIb did not show the signal due to the equatorial C_1–H, thus supporting β-configurational structure of this glucuronide derivative. The free glucuronide (Vb) was obtained on hydrolysis of IIIb. Since the ultraviolet (UV) spectrum of Vb showed a marked bathochromic shift in an alkaline medium as that of morphine (Table I), it is no doubt that it possesses a phenolic hydroxyl group. In the IR spectrum, the existence of the non-ionized carboxyl peak at 1750 cm^{-1} and the strong hydrogen-bonding hydroxyl group around 2640 cm^{-1} suggested that the structure should be best described as Vb. The IR spectra of morphine and 6-acetylmorphine also exhibited this hydrogen-bonding hydroxyl band around 2640 cm^{-1}.

Table I. UV Absorption Maxima and Molecular Extinction Coefficients

Compound	λ_{max} (mμ)	log ε	Solvent
Codeine (Ia)	286	3.25	95% EtOH
Morphine (Ⅵ)	287	3.21	95% EtOH
	298	3.41	0.1 N NaOH
3-Acetylmorphine (Ib) (sulfamate)	282	3.39	H_2O
6-Acetylmorphine	287	3.19	95% EtOH
Methyl [codein-6-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid]uronate (Ⅲa)	287	3.22	95% EtOH
Methyl [3-acetylmorphin-6-yl-2,34-tri-O-acetyl-β-D-glucopyranosid]uronate (Ⅲb)	283	3.35	95% EtOH
Codein-6-yl-β-D-glucopyranosiduronate (Va)	285	3.23	H_2O
Morphin-6-yl-β-D-glucopyranosiduronate (Vb)	286	3.18	H_2O
	298	3.45	0.1 N NaOH
Morphin-3-yl-β-D-glucopyranosiduronate (Ⅷ)	283	3.24	H_2O

An attempt to synthesize morphine-3-glucuronide (VIII) by the condensation of 6-acetylmorphine with acetobromo sugar derivative similar to the above glucuronide synthesis resulted in the formation of resinous material. By the method of Helferich and Berger,[15] in which the condensation was performed in the presence of mercuric cyanide in acetonitrile, formation of the expected glucuronide derivative was demonstrated by thin-layer chromatography. However, the following hydrolytic removal of the protecting groups resulted in a cleavage of glycosidic linkage.

Then, the synthesis of VIII was performed by the employment of sodium hydroxide as the condensing agent in aqueous acetone solution. The method used was essentially the same as those by Mannich[8] and by Casparis and Béchert.[9] However, in this glucuronide synthesis, different from the above glycosides synthesis, pH value of the reaction mixture

15) B. Helferich and A. Berger, *Chem. Ber.*, **90**, 2492 (1957).
16) L.H. Welsh, *J. Org. Chem.*, **19**, 1409 (1954).

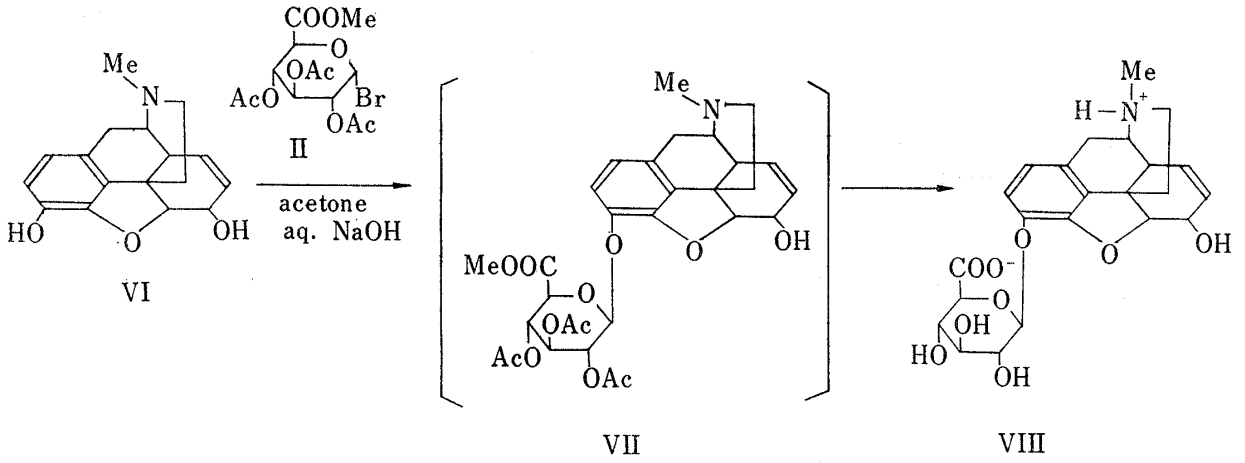

Chart 2

went down rather rapidly[17] and large portion of morphine soon precipitated out from the reaction mixture. Therefore, in order to push the reaction, it was necessary to dissolve the precipitated morphine sometimes and to supply II repeatedly.

The reaction mixture, after allowing to stand over night, formed two layers, aqueous (lower) and acetone (upper). This was probably due to salting out mechanism by sodium bromide produced during the reaction, and seemed very desirable for the reaction process, because the intermediate (VII), which was formed gradually, was distributed more into the acetone layer than the aqueous alkaline layer, and therefore partly protected from the continuous hydrolysis of the glycosidic linkage. It was quickly hydrolyzed on occasional stirring to the free glucuronide (VIII) which was transferred mostly into aqueous layer, but no more unstable against alkali. VIII was thus accumulated gradually in the aqueous layer. The proposed process was supported by the thin-layer chromatographic examination.

After the extraction of the unchanged morphine the reaction mixture was passed through a column of Dowex 50 W-X 8 (H-form) and eluted with 0.15 N NH_4OH to give pure VIII. The strong IR absorption band at 1597 cm^{-1} due to carboxylic ion indicated that VIII exists in an ionized from like codeine glucuronide (Va).

All of these three glucuronides could be hydrolyzed with β-glucuronidase, although the rate was lower in the alcoholic glucuronides (Va,b) than in the phenolic glucuronide (VIII). The results are summarized in Table II.

Table II. Hydrolysis of Codeine and Morphine Glucuronides with β-Glucuronidase

Substrate	Percent of hydrolysis
Codeine glucuronide (Va)	63.5
Morphine-6-glucuronide (Vb)	65.0
Morphine-3-glucuronide (VIII)	92.5

Incubation mixture consists of 0.2 μmoles of substrate in 1 ml of H_2O, 1 ml of β-glucuronidase preparation (7000 p-nitrophenol glucuronide unit/ml), 2 ml of 0.2 M acetate buffer (pH 5.0) and 1 ml of H_2O. It was incubated at 37° for 21 hr. Liberated codeine and morphine were estimated by the method of Yoshimura, *et al.*[18]

Codeine and morphine liberated after the treatment with β-glucuronidase were also identified by thin-layer chromatography. It was found that these three glucuronides were

17) This may be due to a reaction between NaOH and acetobromo derivative of glucuronic acid (II), by which NaOH was consumed.

18) H. Yoshimura, K. Oguri and H. Tsukamoto, *Chem. Pharm. Bull.* (Tokyo), **14**, 62 (1966).

distinguishable with one another by thin-layer chromatography. These *Rf* values are listed in Table III.

Table III. *Rf* Values of Thin-Layer Chromatography of Codeine and Morphine Glucuronides and Their Methyl Acetyl Derivatives

Compound	Solvent systems A	B	C	D
Methyl acetyl derivative of codeine glucuronide (IIIa)	0.6	0.75	—	—
Methyl acetyl derivative of morphine-6-glucuronide (IIIb)	0.54	0.75	—	—
Methyl acetyl derivative of morphine-3-glucuronide (VII)	—	0.75	—	—
Methyl derivative of codeine glucuronide (IVa)	—	0.54	—	—
Methyl derivative of morphine-6-glucuronide (IVb)	—	0.54	—	—
Codeine glucuronide (Va)	—	0	0.28	0.55
Morphine-6-glucuronide (Vb)	—	0	0.24	0.45
Morphine-3-glucuronide (VIII)	—	0	0.17	0.35

solvent systems: A, $CHCl_3$–MeOH (4:1); B, $CHCl_3$–MeOH–H_2O (65:35:10, lower layer); C, BuOH–acetone–AcOH–5% NH_4OH–H_2O (45:15:10:10:20); D, BuOH–AcOH–H_2O (4:1:5, upper layer)
chromatoplates: Silicagel was used for A, B and C, and *MN*–cellulose powder for D.
color reagents: Potassium platinum iodide and ferric hydroxamate reaction were used for A and B, and Dragendorff reagent for C and D.

Above thin-layer chromatography was effectively applied to the examination of urinary metabolites of codeine and morphine. The results will be reported elsewhere in the near future.

Experimental

All melting points were uncorrected. UV spectra were recorded with Shimadzu spectophotometer, model SV-50A. IR spectra were recorded with a JASCO spectrometer, model DS-301, in potassium bromide disks. NMR spectra were measured with a JEOL 3H-60 spectrometer using tetramethylsilane as internal standard.

Thin-Layer Chromatography——Silicagel G (Merck) and *MN*–Cellulose Powder 300G (Macherey, Nagel & Co.) were used for the preparation of chromatoplates, 0.25 mm thickness. They were activated at 105° for 30 min and 15 min, respectively. The solvent systems, color reagents, and *Rf* values are listed in Table III.

3-Acetylmorphine (Ib)——Ib was prepared by the method of Welsh.[16] Morphine (2.12 g) was suspended in 200 ml of 10% $NaHCO_3$ solution, and to this suspension was added 10 ml of acetic anhydride. After ceased to foam, the mixture was poured onto ice water and extracted with $CHCl_3$. The $CHCl_3$ layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated to dryness to give 2.1 g of amorphous Ib.

6-Acetylmorphine——6-Acetylmorphine hydrochloride prepared from 0.86 g of diacetylmorphine by the method of Wright[19] was dissolved in H_2O and extracted with $CHCl_3$–iso-PrOH (3:1) at pH 8.0. The yield was 0.73 g.

Methyl[codein-6-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid]uronate (IIIa)——Codeine monohydrate (Ia, 1.0 g) was dissolved in 200 ml of sodium dried benzene in a four necked flask which was protected from the moisture. To this boiling solution, a solution of 2.5 g of methyl 2,3,4-tri-O-acetyl-1α-bromo-1-deoxy-D-glucopyranuronate (II) in 50 ml of sodium–dried benzene and 2.0 g of freshly prepared dry Ag_2CO_3[20] were added slowly during about 9 hr. In the meantime, benzene was distilled off gradually, and stirring was continued. The formation of reaction product and disappearance of Ia were occasionally checked by thin-layer chromatography (system A, silicagel, see Table III). The solid in the reaction mixture was filtered off after Ia disappeared mostly (about 10 hr) and washed with benzene. The solvent of the combined filtrates was evaporated to dryness *in vacuo*. This viscous residue was crystallized from EtOH and recrystallized from the same solvent to colorless needles, mp 112—114°. The mother liquor was further purified by chromatography on silicagel column using the effluent solvents of benzene, benzene–$CHCl_3$ (1:1), $CHCl_3$

19) C.I. Wright, *J. Pharmacol. Exptl. Therap.*, **71**, 164 (1941).
20) M.L. Wolfrom and D.R. Lineback, "Method in Carbohydrate Chemistry," Vol. 2, ed. by R.L. Whistler and M.L. Wolfrom, Academic Press, New York and London, 1963, p. 341.

and $CHCl_3$–EtOH (19:1). From the fractions, positive to alkaloid reagent, which were eluted with $CHCl_3$–EtOH (19:1), the same crystals of mp 112—114° were also obtained. The yield was 0.53 g. *Anal.* Calcd. for $C_{31}H_{37}O_{12}N \cdot 2H_2O$: C, 57.12; H, 6.34; N, 2.14. Found: C, 56.83; H, 6.45; H, 2.34. IR cm^{-1}: $\nu_{C=O}$ 1765 (KBr). $[\alpha]_D^{26}$ −140° (c=0.5 in $CHCl_3$).

Codein-6-yl-β-D-glucopyranosiduronic Acid (Va)——To 0.9 g of IIIa which was suspended in 5 ml of abs. MeOH was added 3 ml of 1% solution of NaOMe in abs. MeOH. After allowing to stand over night (the completion of solvolysis reaction was indicated by the thin-layer chromatography; system B, silicagel, see Table III), and the reaction mixture was evaporated to dryness *in vacuo*. The residue (IVa) was dissolved in 3.3 ml of 0.43 N $Ba(OH)_2$ and left aside for 1 hr. The barium salt which precipitated on cooling was dissolved in 6 ml of H_2O and adjusted to pH 6.0 with 2 N oxalic acid. The solution was chilled for 1 hr, barium oxalate was removed by filtration, and the filtrate was evaporated to dryness *in vacuo*. The residue was recrystallized from H_2O–MeOH to colorless prisms, mp 276—278° (decomp.). The yield was 0.26 g. *Anal.* Calcd. for $C_{24}H_{29}O_9N \cdot \frac{1}{2}H_2O$: C, 59.50; H, 6.24; N, 2.89. Found: C, 59.46; H, 6.09; N, 2.94. IR cm^{-1}: $\nu_{C=O}$ 1609 (KBr). $[\alpha]_D^{25}$ −216° (c=0.5 in H_2O).

Methyl[3-acetylmorphin-6-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid]uronate (IIIb)——3-Acetylmorphine (Ib) (2.5 g) was condensed with 4.5 g of II in the presence of 4.5 g of Ag_2CO_3 same as the preparation of IIIa. The filtrate of the reaction mixture was concentrated to about 100 ml and extracted with 100 ml of ice-cooled 0.5% HCl for 3 times. Combined extracts were adjusted to pH 8.0 with $NaHCO_3$ and extracted with 200 ml of $CHCl_3$ for 3 times. $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and evaporated to dryness *in vacuo*. The resulting viscous residue was dissolved in EtOH, treated with charcoal and recrystallized from EtOH to colorless prisms, mp 186—188°. The yield was 2.5 g. *Anal.* Calcd. for $C_{32}H_{37}O_{13}N \cdot \frac{1}{2}H_2O$: C, 58.89; H, 5.87; N, 2.15. Found: C, 58.72; H, 5.90; N, 2.24. IR cm^{-1}: $\nu_{C=O}$ 1770 cm^{-1} (KBr). $[\alpha]_D^{26}$ −140° (c=0.5 in $CHCl_3$).

Morphin-6-yl-β-D-glucopyranosiduronic Acid (Vb)——To a suspension of 1 g of IIIb in 5 ml of abs. MeOH, 3.6 ml of 1% NaOMe was added. After treating similarly to the preparation of Va, the residue (IVb) obtained from the reaction mixture was treated with 3.6 ml of 0.43 N $Ba(OH)_2$ and adjusted to pH 6.0 with 2 N oxalic acid. After the removal of barium oxalate by filtration, the filtrate was evaporated to dryness *in vacuo*, and the residue was recrystallized from H_2O–EtOH to colorless needles, mp 254—256° (decomp.). The yield was 0.49 g. *Anal.* Calcd. for $C_{23}H_{27}O_9N \cdot 2H_2O$: C, 55.51; H, 6.28; N, 2.80. Found: C, 55.30; H, 6.69; N, 2.78. IR cm^{-1}: $\nu_{C=O}$ 1750 (KBr). $[\alpha]_D^{28}$ −172° (c=0.5 in H_2O).

Morphin-3-yl-β-D-glucopyranosiduronic Acid (VIII)——To a solution of 1.0 g of morphine (VI) in 3 ml of 2 N NaOH was added a solution of 2.0 g of II in 6 ml of acetone, and the mixture was allowed to stand over night. It formed two layers and precipitated a considerable amount of unchanged VI. With addition of 1 ml of 30% NaOH, this unchanged VI was again dissolved under stirring. To this, a solution of II in 2 ml of acetone was added and the mixture was allowed to stand over night. Such a procedure was further repeated 7 times more (a total of 6.0 g of II was used against 1.0 g of VI). The reaction mixture was adjusted to pH 9.0 with AcOH and extracted 4 times with 50 ml of $CHCl_3$–iso-PrOH (3:1) to remove unchanged VI (600 mg). The aqueous layer was then diluted to 100 ml with H_2O and passed through a column of 150 ml of Dowex 50 W-X 8 (H-form). After washing the resin with 500 ml of H_2O, the glucuronide was eluted with 0.15 N NH_4OH. This chromatography was carried out in a cold room (0°). Combined fractions, positive to Dragendorff reagent, were condensed to about 20 ml, and a trace of VI was extracted 4 times with 30 ml of $CHCl_3$–iso-PrOH (3:1). The aqueous layer was evaporated to dryness, and the residue was recrystallized from H_2O to colorless needles, mp 243—246° (decomp.). The yield was 0.44 g. *Anal.* Calcd. for $C_{23}H_{27}O_9N \cdot 2\frac{1}{2}H_2O$:[21] C, 54.53; H, 6.37; N, 2.76. Found: C, 54.60; 54.69; H, 6.29, 6.34; N, 3.07, 2.78. IR cm^{-1}: $\nu_{C=O}$ 1597 (KBr). $[\alpha]_D^{28}$ −132° (c=0.5 in H_2O).

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21) The sample was dried at 40° for 5 hr *in vacuo*.