The solution were applied for the assay on isolated rabbit duodenum preparation. Two active zones were found, and their *Rf* values were found to correspond to those of the PGE group and PGF group respectively. The extracts, corresponding to the PGE group, were combined, dried and applied to further purification.

Separation of PGE_2 like Substance——The active fraction X which showed PGE like activity was further purified with TLC[6] on silver nitrate impregnated silicagel plate. Fraction X was applied on line with authentic samples of PGE_1 and PGE_2 on both sides. The plate was developed with ethyl acetate–acetic acid–2,2,4-trimethylpentane–water (110:20:30:100). Both reference sides were cut off and the reference spot were detected with 10% alcoholic phosphomolybdic acid. Distribution of smooth muscle stimulating activities on the remaining region of the plate was demonstrated as mentioned above. This procedure gives fraction XII.

Bioassay——The isolated organs were suspended in a bath of controlled temperature, oxigenated with air. The movements were recorded isotonically on a smoked drum with a linear frontal writing lever. Uterus Ringer solution was used for isolated uterus and Tyrode solution for other isolated organs. Compositions of these solutions are as follows: Uterus Ringer solution: NaCl 0.9%, KCl 0.02%, $CaCl_2$ 0.004%, $NaHCO_3$ 0.05% and glucose 0.05%. Tyrode solution: NaCl 0.8%, KCl 0.02%, $CaCl_2$ 0.02%, $MgCl_2$ 0.0093%, $NaHCO_3$ 0.1%, NaH_2PO_4 0.004%, and glucose 0.1%. Experiments *in vivo* were performed on rabbits and rats anesthetized with urethane (2 g/kg body weight *s.c.*). The trachea was canulated and arterial blood pressure was measured by means of mercury manometer.

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On the Structure of Morphine-6-glucuronide in a Solid State

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Recently we have reported the first synthesis of morphine-3- and -6-glucuronides together with the synthesis of codeine glucuronide.[2] It has been subsequently established that both the morphine glucuronides are excreted as the metabolites of morphine in several experimental animals and in human, although the amount of 6-glucuronide excreted is much less than that of 3-isomer.[3,4] For the pharmaceutical study of these glucuronides, a rather large amount of morphine-6-glucuronide became necessary to be synthesized, and therefore several runs of the synthesis of this glucuronide were carried out by the same method as reported previously.[2] However, subsequently obtained crystals of morphine-6-glucuronide (I_b), mp 251—256° (decomp.), exhibited a completely different IR spectrum from that of the former sample (I_a), mp 254—256° (decomp.). This article reports that 6-glucuronide possesses two different structures in a solid state.

The IR spectrum of I_a showed a strong peak at 1750 cm^{-1} which suggested that I_a should exist in the structure possessing a non-ionized carboxyl group shown in Fig. 1. On the other

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[1] Location: *Katakasu, Fukuoka.*

[2] H. Yoshimura, K. Oguri and H. Tsukamoto, *Tetrahedron Letters*, **1968**, 483; *idem, Chem. Pharm. Bull.* (Tokyo), **16**, 2114 (1968).

[3] H. Yoshimura, K. Oguri and H. Tsukamoto, *Biochem. Pharmacol.*, **18**, 279 (1969).

[4] H. Yoshimura, S. Îda, K. Oguri, E. Hasegawa and H. Tsukamoto, Meeting of Kyushu Branch, Pharmaceutical Society of Japan, Fukuoka, July 1968.

hand, that of the crystalline sample of Ib showed a strong peak at 1600 cm^{-1} due to the ionized carboxyl group and broad absorptions between 2800 and 2400 cm^{-1} probably due to the positively charged piperidine nitrogen as shown in Fig. 1.

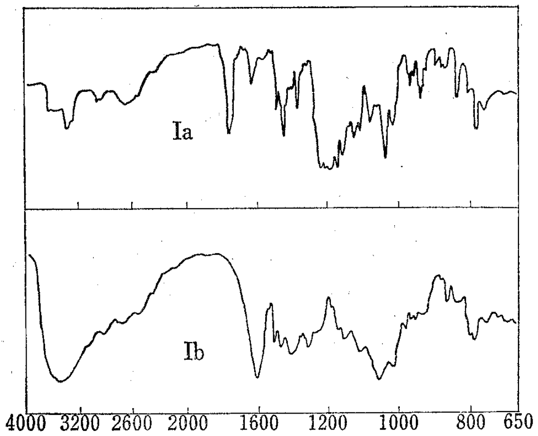
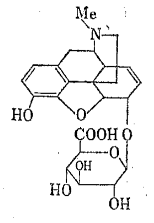
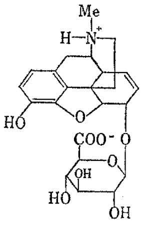


Fig. 1. The IR Absorption Spectra of I_a and I_b (KBr·Disk)



Ia



Ib

All other properties of Ia and Ib, however, are just the same each other. They are listed in Table I. Furthermore, elemental analysis of Ib also afforded the dihydrated formula, same as that of Ia. On the treatment with β-glucuronidase, both Ia and Ib liberated morphine.

Table I. Comparison of Several Properties of Ia and Ib

Properties / Substances	UV λ_{max} mμ (log ε) in H_2O	UV λ_{max} mμ (log ε) in 0.1N NaOH	Rf (TLC)[b]	$[\alpha]_D$ (c=0.5 in H_2O)
I_a[a]	286 (3.18)	298 (3.45)	0.24	−172° (at 28°C)
I_b	286 (3.16)	298 (3.40)	0.24	−166° (at 17°C)

a) The data were quoted from reference 1.
b) solvent system: BuOH–acetone–AcOH–5% NH_4OH–H_2O (45:15:10:10:20)
chromatoplates: Silicagel G (Merck)

From these results, it has become evident that Ia and Ib exist as the non-ionized and ionized forms, respectively, only in a solid state, but they possess the same structure in a solution. In the synthesis of morphine-6-glucuronide, Ia was obtained only once previously but never after. As previously reported, both of morphine-3-glucuronide and codeine glucuronide were crystallized as the ionized form.[2] These facts suggest that Ib is probably more stable form than I_a.

It must be noticed that morphine-6-glucuronide exhibited almost the same analgesic activity as morphine when injected intraperitoneally into mice.[5] The detail of this interesting result will appear elesewhere in the near future.

Experimental

Melting points were uncorrected. The spectra were recorded on the following instruments: IR, JASCO DS-301; UV, Shimadzu SV-50A. Morphine-6-glucuronide (Ib) was synthesized by the wholly same method as previously reported.[2] Colorless needles, mp 251—256° (decomp.). *Anal.* Calcd. for $C_{23}H_{27}O_9N \cdot 2H_2O$ (I_b): C, 55.51; H, 6.28; N, 2.80. Found: C, 55.96; H, 6.61; N, 2.62. The thin-layer chomatoplates were prepared with Silicagel G (Merck) 0.25 mm thick. The solvent system was listed in the footnote of Table I. The enzymatic hydrolysis of I_b was performed by the same method as previously reported.[2]

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5) O. Kamata, S. Watanabe, S. Ishii, S. Ueki, K. Oguri, S. Îda, H. Yoshimura and H. Tsukamoto, Abstracts of Papers, The 89th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April 1969, p. 443.