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155. **Shoji Takitani**: Alkylamine N-Glucuronides and their Stability in the Solution.

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Recently it has been substantiated that some amines in the body are conjugated with glucuronic acid in their metabolic process and excreted into urine in the form of their N-glucuronides. These findings on the formation of N-glucuronides *in vivo* offer an interesting problem not only as to the metabolism of drugs, but also from the pharmacological point of view.

In the previous paper,[1] the preparation of N-glucuronides of several aromatic amines and the evidence that the N-glucuronide obtained has a structure of the amine N-glucopyranosyluronide have been reported.

In the present paper, synthesis of the N-glucuronides of several aliphatic amines and measurement of the dissociation constant of aryl- and alkyl-amine N-glucuronides are presented with reference to their stabilty in the biological system.

I. Preparation of the Alkylamine N-Glucuronide

Aliphatic primary amines gave their N-glucuronide easily when they were condensed with glucuronic acid or its sodium salt in aqueous solution at room temperature. Thus, a series of N-glucuronides were prepared in fairly good yield by the following procedure: Alkylamine (3~5 mol. equiv.) was added to a concentrated aqueous solution of sodium glucuronate. The mixture was left overnight at room temperature, shielded from light. The product was precipitated by adding methanol (or ethanol, acetone) to

Table I. Alkylamine N-Glucuronides

Compd. No.	Alkylamine N-glucuronide	m.p. (°C) (decomp.) (uncorr.)	$[\alpha]_D$ (l=1, t=10°, H_2O)	Empirical formula	Analysis (%)	C	H	N	Na	Yield (%)	Crystal form
(I)	Na benzylamine N-glucosyluronate	117~118	−62.01→−24.23 (c=3.47, t=24°C)	$C_{13}H_{16}O_6NNa\cdot\frac{1}{2}H_2O$	C. F.	49.68 49.24	5.45 4.99	4.46 4.51	7.32 7.38	53	A
(II)	Na phenethylamine N-glucosyluronate	126~127	−26.70→−15.60 (c=2.50)	$C_{14}H_{18}O_6NNa\cdot1\frac{1}{2}H_2O$	C. F.	48.54 48.59	6.11 5.94	4.05 4.14	6.64 6.49	55	B
(III)	Na ethylamine N-glucosyluronate	106~110	−29.35→−10.47 (c=5.35)	$C_8H_{14}O_6NNa\cdot\frac{1}{2}H_2O$	C. F.	38.11 37.85	6.00 6.46	5.55 5.24	9.12 8.73	60	B
(IV)	Na isopropylamine N-glucosyluronate	109	−30.00→0 (c=3.00)	$C_9H_{16}O_6NNa\cdot1\frac{1}{2}H_2O$	C. F.	38.03 37.84	6.74 6.89	4.93 5.08	8.09 7.95	66	A
(V)	Na butylamine N-glucosyluronate	120	−33.33→−8.33 (c=3.00)	$C_{10}H_{18}O_6NNa\cdot H_2O$	C. F.	41.51 41.37	6.97 6.69	4.84 5.11	7.95 7.98	62	A
(VI)	Na isobutylamine N-glucosyluronate	119	−36.33→−8.33 (c=3.00)	$C_{10}H_{18}O_6NNa\cdot1\frac{1}{2}H_2O$	C. F.	40.26 40.33	7.10 7.22	4.70 4.70	7.71 7.80	73	A
(VII)	Na cyclohexylamine N-glucosyluronate	108~109	−35.33→−2.00 (c=3.00)	$C_{12}H_{20}O_6NNa\cdot1\frac{1}{2}H_2O$	C. F.	44.44 44.35	6.84 7.02	4.32 4.43	7.10 7.03	56	A
(VIII)	Addition product of benzylamine N-glucuronic acid and benzylamine	116	−21.33→−14.00 (c=3.00)	$C_{20}H_{25}O_6N_2\cdot H_2O$	C. F.	58.95 58.77	6.68 6.72	6.87 6.73	— —	66	B
(IX)	Addition product of isobutylamine N-glucuronic acid and isobutylamine	99~101	−15.00→0 (c=3.00)	$C_{14}H_{30}O_6N_2\cdot1\frac{1}{2}H_2O$	C. F.	48.12 48.13	9.50 9.08	8.02 8.14	— —	77	B

C.: Calcd. F: Found A: fine needles B: leaflets

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1) M. Ishidate, *et al.*: This Bulletin, **7**, 291(1959).

this solution. Recrystallization by precipitating the product from aqueous solution with methanol or ethanol on cooling afforded fine needles or leaflets. By this procedure, crystalline N-glucuronides were prepared with benzylamine, phenethylamine, ethylamine, isopropylamine, butylamine, isobutylamine, and cyclohexylamine.

On the other hand, when glucuronic acid was used instead of its sodium salt, benzylamine and isobutylamine gave an addition product composed of one mole each of the amine and its N-glucuronide, as in the case of aromatic amines.[1] The properties of the N-glucuronide and the addition product described above are shown in Table I.

II. Degree of Dissociation and Dissociation Constants of N-Glucuronides

By measuring optical rotation, the degree of dissociation and the dissociation constant of alkylamine and arylamine N-glucuronides were determined according to the following equation, supposing that the amine ($R\text{-}NH_2$) and sodium glucuronate form sodium amine N-glucosyluronate as shown below.

$$\underset{C(n-1+\alpha)}{R\text{-}NH_2} + \underset{C\alpha}{HO\text{-}\underbrace{CH\text{-}(CHOH)_3\text{-}CH}_{O}\text{-}COO^-} \rightleftarrows \underset{C(1-\alpha)\ \text{equilibrium concentration}}{R\text{-}NH\text{-}\underbrace{CH\text{-}(CHOH)_3\text{-}CH}_{O}\text{-}COO^-}$$

The dissociation constant (K) and the degree of dissociation (α) may be written as :

$$K=\frac{(n-1+\alpha)C\alpha}{1-\alpha}$$

$$R_D=[R_G]_D\,C\alpha+[R_{NG}]_D\,C(1-\alpha)$$

$$\alpha=\frac{R_D-[R_{NG}]_D\,C}{[R_G]_D\,C-[R_{NG}]_D\,C}$$

where R_D : Rotation for a solution of $R\text{-}NH_2$ and Na glucuronate ($n=1$) at the equilibrium.

$[R_G]_D$: Rotation of $1M$ soln. of Na glucuronate.

$[R_{NG}]_D$: Rotation of $1M$ soln. of Na amine N-glucosyluronate when dissociation does not occur by adding excess RNH_2 to Na glucuronate.

n : Molar ratio of $R\text{-}NH_2$: Na glucuronate

In general, the reaction proceeded easily in dilute solution ($0.1M$) at room temperature and the equilibrium was reached in 3～4 hours. The dissociation constant (K) of several N-glucuronides, calculated according to the equation described above and typical examples of the rotation curve of aryl- and alkyl-amine N-glucuronides are shown in Table II and Figs. 1 and 2, respectively.

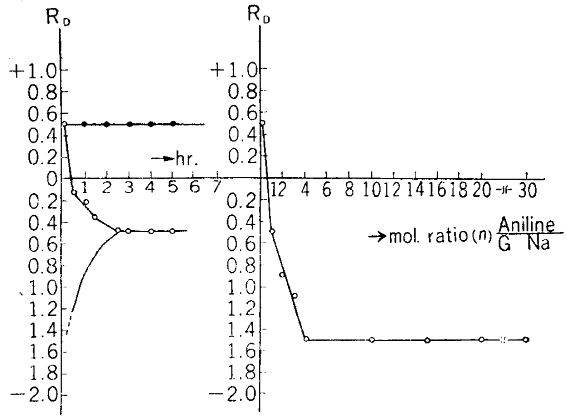

Fig. 1.

Aniline + Na glucuronate ($0.1M$ soln.)

AcOH-AcONa; 30% EtOH, pH 5.5

Aniline : G Na	α	K
1 : 1	0.50	5.0×10^{-2}
2 : 1	0.30	5.6×10^{-2}
3 : 1	0.20	5.0×10^{-2}

–•– Na glucuronate

–○– aniline + Na glucuronate

—— Na aniline N-glucosyluronate

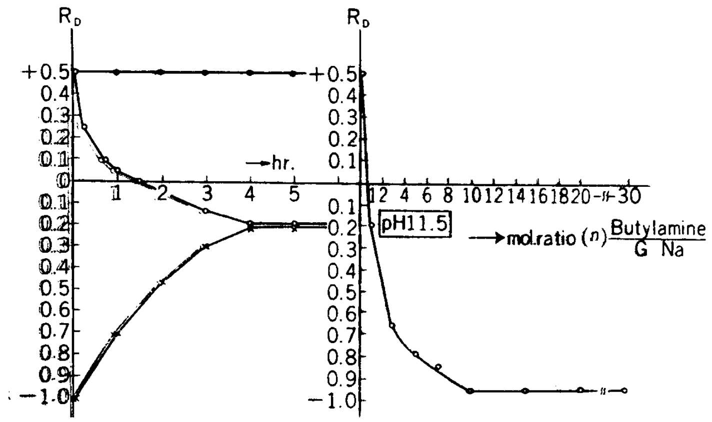

Fig. 2.

Butylamine + Na glucuronate (0.1M soln.)

Butylamine : G Na	α	K
1 : 1	0.52	5.8×10^{-2}
3 : 1	0.21	5.9×10^{-2}
5 : 1	0.12	5.6×10^{-2}
7 : 1	0.08	5.5×10^{-2}

(at 10°C)

-•- Na glucuronate
-◦- Butylamine+Na glucuronate
-×- Na butylamine N-glucosyluronate

Table II. Dissociation Constant (K) of N-Glucuronides

N-Glucuronide	K	pH
Na ethylamine N-glucosyluronate	3.50×10^{-2}	11.8
Na isopropylamine N-glucosyluronate	1.09×10^{-1}	11.6
Na butylamine N-glucosyluronate	5.80×10^{-2}	11.5
Na cyclohexylamine N-glucosyluronate	1.07×10^{-1}	11.5
Na aniline N-glucosyluronate	5.0×10^{-2}	5.5
	(at 10°)	

The time needed to reach the equilibrium depends upon pH of the solution. Therefore, when the pH decreased, the solution of the N-glucuronide reached equilibrium rapidly while dissociation was much greater.

That N-glucuronides of alkylamines dissociate more than those of arylamines at the same pH is evident from the comparison of the degree of dissociation of sodium butylamine and sodium aniline N-glucosyluronate at various pH, as indicated in Table III.

Table III. Relationship between pH and Degree of Dissociation (α)

Na butylamine N-glucosyluronate		Na aniline N-glucosyluronate		
pH	α	pH	α	Time to reach the equilibrium (hrs.)
4.4	1.00	4.1	0.59	0.5
5.5	1.00	5.5	0.50	2.5
		6.0	0.50	2.5
7.3	0.66	7.4	0.30	(5～6)×24
8.5	0.62			
9.9	0.54			
11.6	0.52			(at 10°)

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Experimental

I. Preparation of Alkylamine N-Glucuronide

Sodium Benzylamine N-Glucosyluronate (I)—A mixture of benzylamine (3 g.) and aqueous solution (7 cc.) of Na glucuronate (2 g.) was left overnight at 10°. The product separated by adding EtOH: Me_2CO (1:1) was washed with EtOH and Et_2O. Recrystallization by precipitating the product from aqueous solution with EtOH:Me_2CO (1:1) gave fine needles, m.p. 117～118°(decomp.); yield, 1.6 g. Circular paper chromatography using solvent system of PrOH:n-BuOH:0.2N NH_4OH (2:1:1) gave a ring of Rf 0.6 which was located by both ninhydrin and aniline hydrogen phthalate reagents.

Compounds (II)~(IX) were prepared by this method and the properties of these compounds with their analytical results are shown in Table I.

II. Measurement of the Degree of Dissociation (α) and Dissociation Constant (K)—All amines used in this study were extra pure grade and Na glucuronate was recrystallized from hydr. MeOH. Rotation tube of 1 dm. long was used in this experiment.

Solution of N-glucuronide for the rotation measurement was prepared by dissolving 0.234 g.(10^{-3} mole) of Na glucuronate monohydrate and the corresponding amine (mol. equiv. for Na glucuronate) in water and diluting this solution to a final volume of 10 cc. with water. In the case of aniline, the buffer solution of AcOH and AcONa in 30% EtOH of various pH was used because of the low solubility of aniline in H_2O. The rotation of this solution (A) was measured at various time intervals until the equilibrium of the solution was reached, and the rotation of 0.1M solution of Na amine N-glucosyluronate (B) was measured in the same way. The values of rotation (A) and (B) thus determined agreed with each other after several hours as indicated on the left side of Figs. 1 and 2.

On the other hand, rotation of the solution of Na amine N-glucosyluronate at the equilibrium was measured when its dissociation was suppressed by adding an excess of the amine as shown on the right side of Figs. 1 and 2.

The amines used in the present study did not show any rotation. Na glucuronate showed mutarotation in the solution, but it changed quite rapidly to reach the constant value ($[R_G]_D=5.0$) in various pH. From these results, R_D and $[R_{NG}]_D$ could be calculated.

Therefore, the degree of dissociation (α) and the dissociation constant (K) of several Na amine N-glucosyluronates prepared in this work could be calculated from the above equation (cf. Tables II and III, Figs. 1 and 2).

For example, α and K of Na butylamine N-glucosyluronate were determined by the following calculations :

$$C=0.1, \quad [R_{NG}]_D=-9.5, \quad R_D=-0.2, \quad n=1$$

$$\alpha=\frac{-0.2-(-9.5)\times0.1}{5\times0.1-(-9.5)\times0.1}=0.52$$

$$K=\frac{0.1\times(0.52)^2}{1-0.52}=5.8\times10^{-2} \qquad \text{(at 10°, pH 11.5)}$$

Summary

(1) The N-glucuronides of primary aliphatic amines, i.e. benzylamine, phenethylamine, ethylamine, isopropylamine, butylamine, isobutylamine, and cyclohexylamine, were prepared.

(2) The degree of dissociation and dissociation constants of sodium aryl- and alkylamine N-glucosyluronate were determined by measuring their optical rotation.

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