[Chem. Pharm. Bull.
14(1) 22～29 (1966)]

UDC 615.41-014 : 547.581.2.09

5. **Seigo Ueda** : The Mechanism of Solubilization of Water Insoluble Substances with Sodium Benzoate Derivatives. I. The Interaction between Water Insoluble Substances and Sodium Benzoate Derivatives in Aqueous Solution.

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In pharmaceutical field, it is required to prepare the aqueous solutions of a variety of water insoluble drugs. This has been achieved by addition of the so-called solubilizing agents which may be exemplified by sodium benzoates or nicotinamide. Lots of investigations have been reported on these phenomena.[1~4] But no strong evidence has yet been obtained on the solubilization mechanism, although it has previously been speculate that solubilization might be caused by complex formation between insoluble drug molecules and the solubilizing agents added.

This paper concerns spectrophotometric study on this problem and one to one complex-formation between solubilized and solubilizing molecules in water could be found using sodium benzoate derivatives.

Experimental

Materials

Materials studied were so selected that characteristic absorptions of molecular complexes produced were not overlapped with those of parent compounds used. The following compounds were synthesized according to the authentic preparative methods (Melting points are given in parentheses.).

pyrazinamide (190°)
2-aminotropone (107°)
2-acetamidotropone (111°)
2-benzamidotropone (126°)
cyclohepta[*b*]pyrrol-2(1*H*)-one (164°)
cycloheptimidazol-2(1*H*)-one (245°)
1-benzylcycloheptimidazol-2(1*H*)-one (181°)

Riboflavin was commercially available preparation, according to the Japanese Pharmacopeia VII. Sodium benzoate derivatives were G.R. grade-chemicals purchased from Tokyo Kasei Co., Ltd.

Spectrum Measurements

Visible absorption spectra were measured by a Hitachi automatic recording spectrophotometer (Model EPS 2U) at 25±0.1° and 60±0.1°. The sample solutions were prepared with the phosphate buffered solution of pH 7.0 (0.05 mole/L.).

Results and Discussion

The visible spectra of the drugs to be solubilized are reproduced in Fig. 1. The measured solutions buffered at pH 7.0 contained sodium *p*-aminosalicylate (NaPAS) at various concentrations as indicated in the figure. Spectral changes observed in each case can be considered as due to the special molecular interactions between solubilized and solubilizing components since NaPAS has no absorption in region over

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[*1] Hiromachi, Shinagawa-ku, Tokyo (上田省吾).

1) R. Yamamoto, S. Fujisawa, H. Tanaka : Ann. Repts. Shionogi Research Lab., **5**, 619 (1955).

2) T. Higuchi, *et al.* : J. Am. Pharm. Assoc., **41**, 10 (1952); **42**, 132 (1953); **42**, 138 (1953); **43**, 527 (1954); **46**, 458 (1957); **46**, 587 (1957); **50**, 905 (1961).

3) M. Samejima : Yakugaku Zasshi, **80**, 92 (1960); **80**, 95 (1960); **89**, 99 (1960); **80**, 1706 (1960); **80**, 1713 (1960).

4) K. Sekiguchi : *Ibid.*, **81**, 664 (1961).

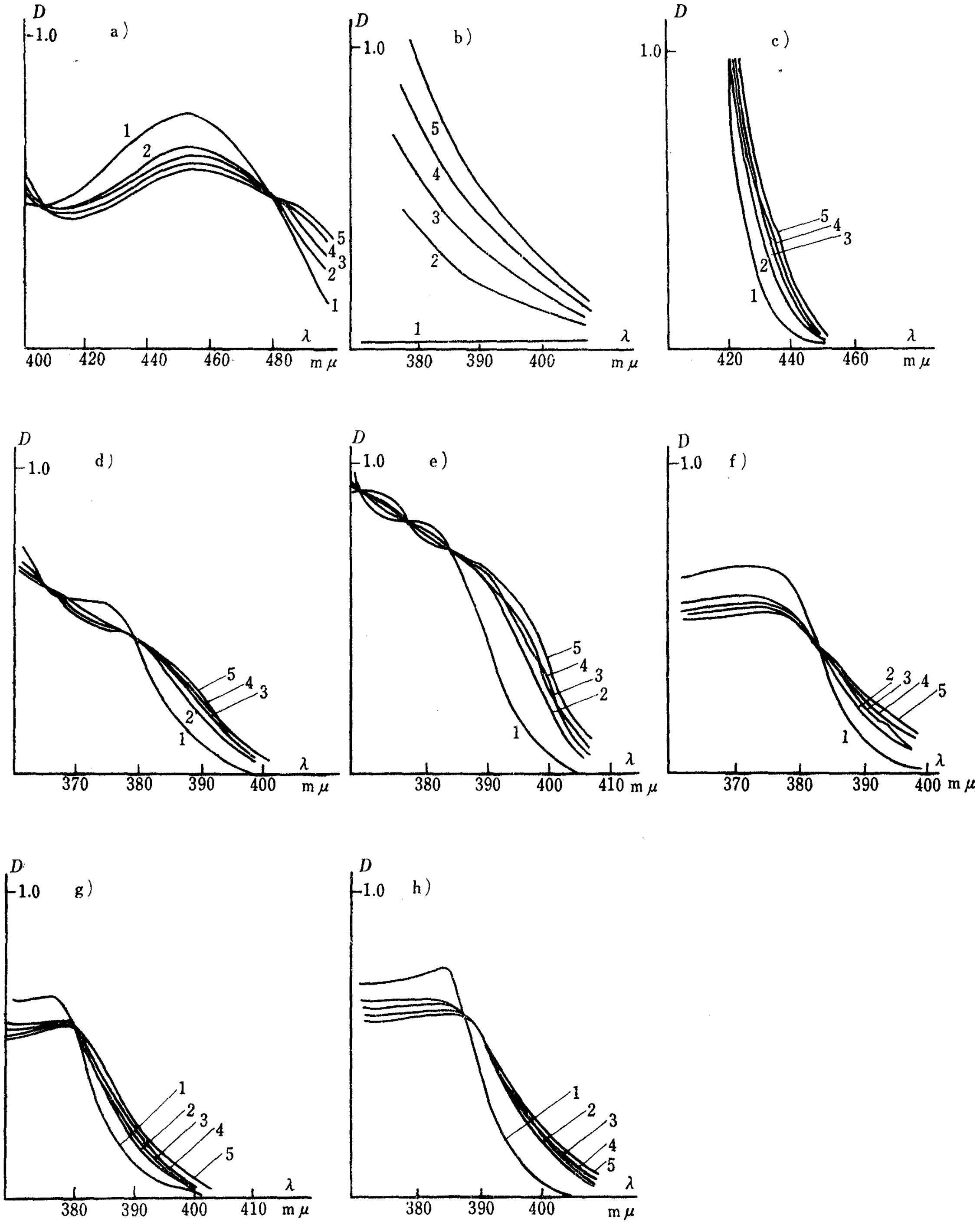

Fig. 1. The Visible Absorption Spectra of the Aqueous Solution of *p*-Aminosalicylate and Water Insoluble Substances

a) Riboflavine 1.7×10^{-4} mol./L.
b) Pyrazinamide 4.06×10^{-2} mol./L.
c) 2-Aminotropone 8.2×10^{-3} mol./L.
d) 2-Acetamidotropone 9.2×10^{-4} mol./L.
e) 2-Benzamidotropone 1.03×10^{-3} mol./L.
f) Cyclohepta[*b*]pyrrol-2(1*H*)-one 1.03×10^{-3} mol./L. 0.1 cm. cell
g) Cycloheptimidazol-2(1*H*)-one 1.0×10^{-3} mol./L. 0.1 cm. cell
h) 1-Benzylcycloheptimidazol-2(1*H*)-one 1.06×10^{-3} mol./L. 0.1 cm. cell

1: Water insoluble substance only
2: NaPAS 0.4 mol./L.
3: NaPAS 0.6 mol./L.
4: NaPAS 0.8 mol./L.
5: NaPAS 1.0 mol./L.

370 mμ. Analogous spectral changes were brought about by addition of other sodium benzoate derivatives used in the present study.

The fact that isosbestic points appeared in most cases as shown in Fig. 1 may probably indicate existence of only single species of complex. Then, the contineous variation method[5] which is uaually very effective to determine the constitution of the complex was applied to the case of 2-acetamidotropone and cycloheptimidazol-2(1*H*)-one with NaPAS or sodium salicylate. The results, as shown in Fig. 2, gave a strong evidence of one to one complex formation in these cases.

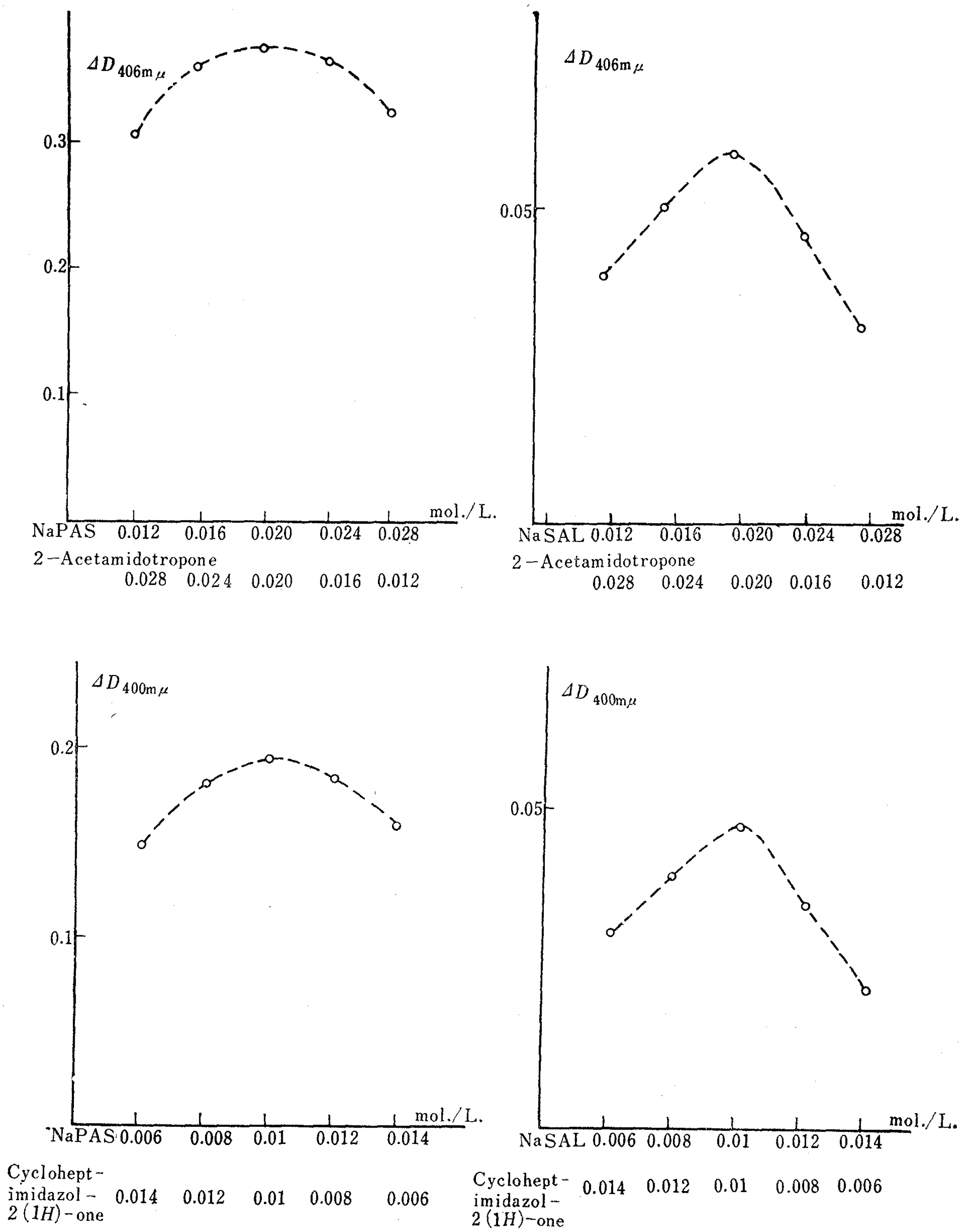

Fig. 2. The Plots by the Contineous Variation Method

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5) A.E. Harrey : J. Am. Chem. Soc., 72, 4488 (1950).

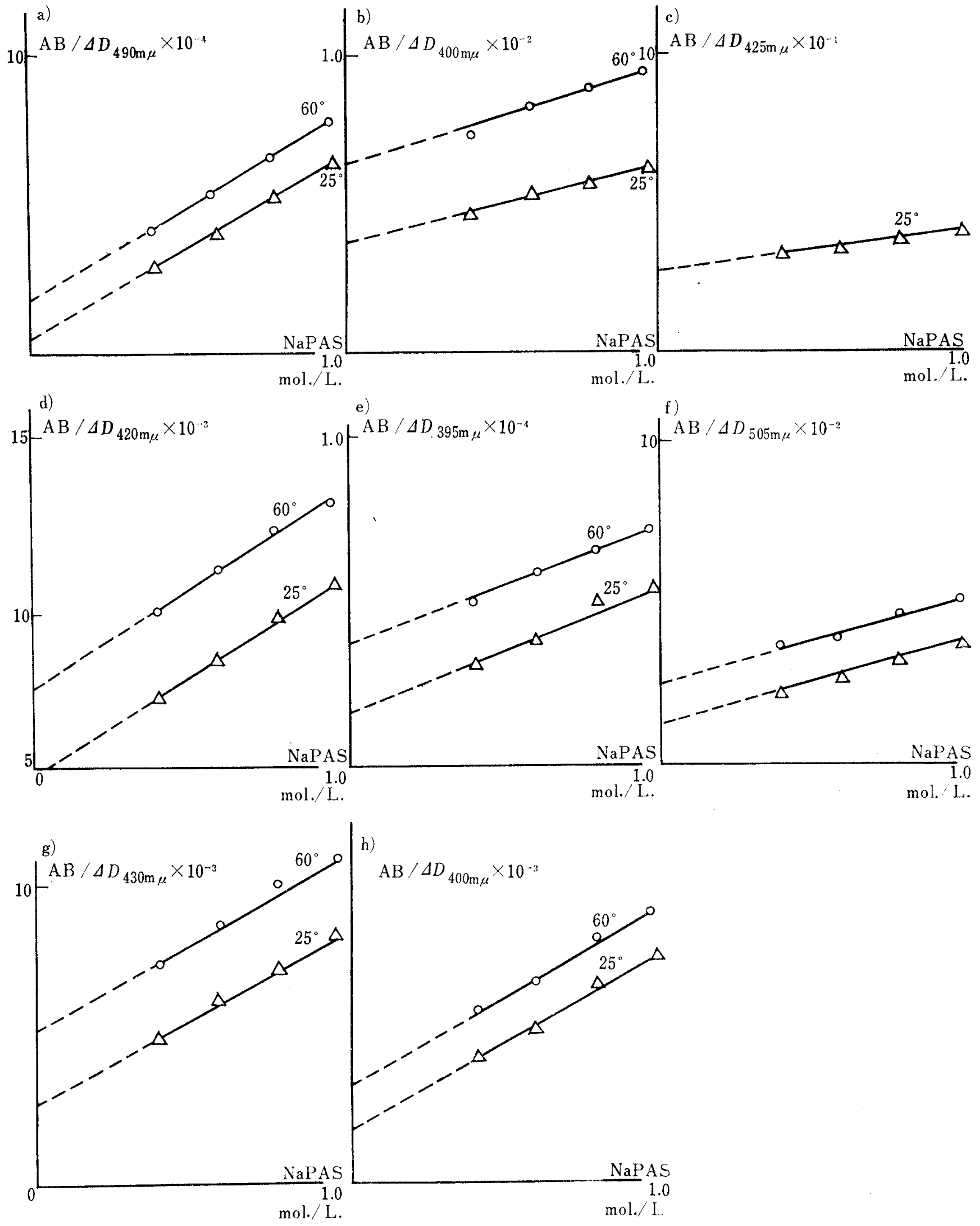

Fig. 3. The Plots by the Benesi-Hildebrand Method

a) Riboflavine 1.0×10^{-4} mol./L.
b) Pyrazinamide 4.06×10^{-2} mol./L.
c) 2-Aminotropone 8.25×10^{-3} mol./L.
d) 2-Acetamidotropone 6.13×10^{-3} mol./L.
e) 2-Benzamidotropone 1.0×10^{-4} mol./L.
f) Cyclohepta[*b*]pyrrol-2(1*H*)-one 6.85×10^{-3} mol./L.
g) Cycloheptimidazol-2(1*H*)-one 6.85×10^{-3} mol./L.
h) 1-Benzylcycloheptimidazol-2(1*H*)-one
1.06×10^{-3} mol./L.

From the above observations, it can be considered that the conclusion of one to one complex formation may be generalized to all systems composed of solubilized and solubilizing solutes, although experimental proof was obtained in restricted number of systems. On this assumption, the stoichiometrics, that is, the formation constants Ks and molar extinction coefficients ϵs of the complexes were tried to be determined. Thus the concentration-dependent spectral changes such as shown in Fig. 1 were analysed according to the Benesi-Hildebrand method6) to show the linear relationship between $(A)(B)/D$ and (B), as shown in Fig. 3, where (A), (B), and D were molar concentration of solubilized and solubilizing components added and observed absorbance of the mixed solution, respectively. This is a further strong support on one to one complex-formation in these systems. Molar extinction coefficients ϵs and formation constnats Ks of the complexes were evaluated from the slope and the intercepts of the Benesi-Hildebrand plots, as shown in Fig. 3, respectively. They are shown in Table I to Ⅷ. Using K values thus obtained, enthalpy and enthropy changes, ΔH and ΔS, of complex formations were also obtained by equation (1), which are listed in the same tables.

$$-RT \ln K = \Delta H - T\Delta S \qquad (1)$$

It has now become revealed that enthropy changes for all of the systems studied decreased in complex-formation processes, and that molecular extinction coefficients of the complexes did not depend on temperature in all of the cases. As a results,

TABLE I. ϵ, K, ΔH and ΔS of the Complex Formation of Riboflavine

Sodium benzoate deriv.	λ (mμ)	25° ϵ	25° K	60° ϵ	60° K	ΔH (kcal./mole)	ΔS e.u.
Sodium benzoate	490	6280	3.4	6110	0.94	−7.2	−21.8
p-CH_3	490	4780	5.0	4700	1.5	−6.7	−19.3
o-NH_2	490	5260	3.3	5260	1.46	−4.6	−12.9
m-NH_2	490	6050	2.4	6150	1.2	−3.9	−11.4
p-NH_2	500	2180	6.8	2200	2.4	−5.9	−15.8
o-OH	490	5600	7.3	5500	2.4	−6.3	−17.0
m-OH	490	5240	2.8	5040	1.3	−4.3	−12.4
p-OH	490	5240	4.6	5040	2.0	−4.7	−12.7
NaPAS	490	7100	10.7	7100	3.9	−5.7	−12.6

TABLE II. ϵ, K, ΔH and ΔS of the Complex Formation of Pyrazinamide

Sodium benzoate deriv.	λ (mμ)	25° ϵ	25° K	60° ϵ	60° K	ΔH (kcal./mole)	ΔS e.u.
Sodium benzoate	—	—	~0	—	~0	—	—
p-CH_3	350	5.8	0.38	—	—	—	—
o-NH_2	400	89	0.26	88	0.17	−2.4	−10.7
m-NH_2	390	53	0.26	50	0.19	−1.8	− 8.6
p-NH_2	390	100	0.22	100	0.15	−2.2	−10.3
o-OH	366	11	0.44	12	0.25	−3.2	−12.3
m-OH	362	34	0.25	29	0.18	−1.9	− 8.9
p-OH	355	35	0.25	35	0.21	−1.0	− 6.0
NaPAS	400	39	0.78	39	0.52	−2.3	− 6.4

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6) H. A. Benesi, J. H. Hildebrand: J. Am. Chem. Soc., **71**, 270 (1949).

TABLE III. ϵ, K, ΔH and ΔS of the Complex Formation of 2-Aminotropone

Sodium benzoate deriv.	λ (mμ)	25°		60°		ΔH (kcal./mole)	ΔS e.u.
		ϵ	K	ϵ	K		
Sodium benzoate	—	—	~0	—	—	—	—
p-CH_3	420	29	0.2	—	—	—	—
o-NH_2	425	15.5	0.17	—	—	—	—
m-NH_2	420	12	0.3	—	—	—	—
p-NH_2	420	10	0.4	—	—	—	—
o-OH	423	11	0.66	—	—	—	—
m-OH	422	12	0.3	—	—	—	—
p-OH	420	32	0.3	—	—	—	—
NaPAS	420	300	0.91	—	—	—	—

TABLE IV. ϵ, K, ΔH and ΔS of the Complex Formation of 2-Acetamidotropone

Sodium benzoate deriv.	λ (mμ)	25°		60°		ΔH (kcal./mole)	ΔS e.u.
		ϵ	K	ϵ	K		
Sodium benzoate	405	118	0.06	—	—	—	—
p-CH_3	400	428	0.35	—	—	—	—
o-NH_2	410	400	0.56	400	0.33	−3.0	−11.1
m-NH_2	400	505	0.30	535	0.20	−2.0	−10.0
p-NH_2	410	418	0.50	457	0.31	−2.7	−10.4
o-OH	401	171	1.75	183	0.50	−7.0	−22.4
m-OH	405	394	0.31	419	0.25	−1.2	−6.3
p-OH	405	188	0.73	195	0.61	−1.0	−4.0
NaPAS	420	143	1.7	148	1.0	−3.0	−8.9

TABLE V. ϵ, K, ΔH and ΔS of the Complex Formation of 2-Benzamidotropone

Sodium benzoate deriv.	λ (mμ)	25°		60°		ΔH (kcal./mole)	ΔS e.u.
		ϵ	K	ϵ	K		
Sodium benzoate	390	6600	0.75	6550	0.47	−2.6	−9.2
p-CH_3	395	6700	0.74	6800	0.47	−2.5	−9.1
o-NH_2	394	6700	0.87	6700	0.55	−2.6	−11.3
m-NH_2	394	9000	0.96	8700	0.55	−1.4	−4.7
p-NH_2	395	10000	1.4	10000	0.75	−3.5	−12.5
o-OH	395	4600	2.2	4500	0.96	−4.7	−14.0
m-OH	390	7700	1.4	7700	0.48	−6.1	−19.8
p-OH	400	3900	2.4	3900	0.89	−5.6	−17.0
NaPAS	400	2300	2.2	2400	1.3	−3.0	−8.3

TABLE VI. ϵ, K, ΔH and ΔS of the Complex Formation of Cyclohepta[b]pyrrol-2(1H)-one

Sodium benzoate deriv.	λ (mμ)	25°		60°		ΔH (kcal./mole)	ΔS e.u.
		ϵ	K	ϵ	K		
Sodium benzoate	504	111	0.09	—	—	—	—
p-CH_3	502	80	0.33	—	—	—	—
o-NH_2	495	154	0.91	160	0.51	−3.2	−11.0
m-NH_2	494	148	0.88	150	0.46	−3.7	−12.5
p-NH_2	495	133	1.04	110	0.57	−3.4	−11.3
o-OH	495	130	2.40	132	0.80	−6.2	−19.0
m-OH	504	80	2.70	82	1.90	−2.0	−6.1
p-OH	500	130	1.30	133	1.03	−1.5	−4.4
NaPAS	505	74	2.0	75	1.14	−3.4	−9.9

Table VII. ϵ, K, ΔH and ΔS of the Complex Formation of Cycloheptimidazol-2(1*H*)-one

Sodium benzoate deriv.	λ (mμ)	25°		60°		ΔH (kcal./mole)	ΔS e.u.
		ϵ	K	ϵ	K		
Sodium benzoate	400	115	0.17	—	—	—	—
p-CH_3	400	410	0.25	—	—	—	—
o-NH_2	410	118	1.44	114	0.75	−3.7	−11.6
m-NH_2	405	220	0.87	200	0.59	−2.2	−7.7
p-NH_2	405	218	1.81	227	0.72	−3.2	−16.2
o-OH	398	1111	1.57	114	1.02	−2.4	−7.3
m-OH	400	483	0.87	84	0.48	−3.3	−11.5
p-OH	400	32	0.98	33	0.66	−2.2	−7.5
NaPAS	410	162	2.67	165	1.28	−4.1	−11.8

Table VIII. ϵ, K, ΔH and ΔS of the Complex Formation of 1-Benzylcycloheptimidazol-2(1*H*)-one

Sodium benzoate deriv.	λ (mμ)	25°		60°		ΔH (kcal./mole)	ΔS e.u.
		ϵ	K	ϵ	K		
Sodium benzoate	400	636	0.29	—	—	—	—
p-CH_3	400	1220	0.42	—	—	—	—
o-NH_2	410	800	1.07	410	817	−2.2	−7.4
m-NH_2	405	720	1.11	405	740	−1.9	−6.0
p-NH_2	405	910	1.05	405	945	−2.4	−7.8
o-OH	398	715	2.15	—	—	—	—
m-OH	400	805	0.87	400	811	−1.2	−4.2
p-OH	400	688	1.16	400	700	−1.4	−4.5
NaPAS	400	670	3.1	410	690	−3.4	−9.1

this led us to an unequivocal conclusion of formation of only one species of one to one molecular complex.⁷⁾ In addition, it may be concluded that complex formation may be attributed to charge transfer force or hydrogen bonding since enthalpy changes were shown to be a few kcal./mol.

It is to be noted that the formation constants, *Ks*, tend to be increased by introduction of such a substituent as CH_3, OH or NH_2 to the aromatic nucleus although they were shown to be close to zero with sodium benzoate itself. Among them, *o*-OH group was most effective. The details of these substituent effects are now under investigation in connection with the structure of the complex produced.

Among the solubilized materials used in the present study, riboflavin was shown to give the most stable complexes with any of the benzoate derivatives, followed by 2-benzamidotropone, cyclohepta[*b*]pyrrol-2(1*H*)-one, cycloheptimidazol-2(1*H*)-one, 1-benzylcycloheptimidazol-2(1*H*)-one and then, pyrazinamide, 2-aminotropone, 2-acetamidotropone.

The author is indebted to Professor H. Nogami of the University of Tokyo for his kind reviewal of this paper before publication and to Dr. T. Kimura, director of this laboratory, for permission for publication of this paper. Thanks are also due to Drs. H. Negoro, E. Awata and T. Miki of this laboratory for their encouragements and discussions throuhout this work.

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7) S.P. Mcglynn : Chem. Revs., 58, 1113 (1958).

Summary

The interactions between eight water insoluble substances and sodium benzoate derivatives in aqueous solutions were studied spectrophotometrically. It was found that the water insoluble substance used formed one to one molecular complex with sodium benzoate derivative in aqueous system. The formation constants of the complexes were determined at 25° and 60°, with which the enthalpy change ΔH and the entropy change ΔS of complex formation were obtained. As a result, it was suggested that the driving force of the complex formation would be due to charge transfer force or hydrogen bonding.

(Received June 2, 2, 1965)

[Chem. Pharm. Bull. 14(1) 29~38 (1966)]

UDC 615.41-014 : 547.581.2.09

6. Seigo Ueda : The Mechanism of Solubilization of Water Insoluble Substances with Sodium Benzoate Derivatives. II.[*1] Solubilities of Water Insoluble Substances in Aqueous Sodium Benzoates Solutions.

(*Product Development Laboratories, Sankyo Co., Ltd.*[*2])

In the previous paper,[*1] it was reported that various water insoluble substances formed molecular complexes with sodium benzoates in aqueous systems. The water insoluble substances cited there were all solubilized with sodium benzoates to various extents.

In this paper, the results of the solubility-measurements of the water insoluble substances in the aqueous solutions containing the sodium benzoates were described and some discussions were made on solubilizing mechanism.

Experimental

Materials

Water insoluble substances and sodium benzoate derivatives used in this paper were the same as described in the Part I of this serial studies.

Solubility Measurement

The solutions of sodium benzoates were prepared with 0.05 mol./L. phosphate buffered solution of pH 7.0. The concentrations were adjusted to 0.5 and 1.0 mol./L. with each solubilizing agents. An excess amounts of water insoluble substances was sealed in a ca. 5 ml. ampoule with about 4 ml. of the sodium benzoates solution prepared above and was agitated at 25°±0.1° for 8 hr. After solubility equilibrium thus obtained, excess amout of the crystal was filtered off. The filtrate was diluted with the phosphate buffered solvent and its UV absorbance D was measured by a Hitachi Model DU spectrophotometer at the wave length indicated in Table I, referring to the solubilizing solvent used in respective cases.

The solubilities were determined from the D values compared with $E^{1\%}_{1\,\mathrm{cm}}$s listed in Table I, since it is reasonably conceivable that a spectral change due to molecular complex formation between a solubilizing and a solubilized agent is completely negligible in such diluted solutions as in the present experimental conditions.

[*1] Part I : This Bulletin, **14**, 22 (1966).

[*2] Hiromachi, Shinagawa-ku, Tokyo (上田省吾).