**Summary**

It was ascertained by paper partition chromatography, ultraviolet spectrum, and bioassay with *L. casei* that the green fluorescent substance, G compound, isolated from the mycelium of *Er. ashbyii* readily reacts with diacetyl or with acetoin to form riboflavin. These results established the structure 8-ribityl-6,7-dimethyllumazine, formerly proposed by the author for the G compound.

On the other hand, a large quantity of acetoin was detected in the mycelium and the culture filtrate, and comparing the amount with those of riboflavin and G compound in these materials, the assumption was made that the biosynthesis of riboflavin by *Er. ashbyii* might be effected by condensation of acetoin with G compound at the position of the methyl groups.

(Received December 24, 1956)

---

*U.D.C. 547.775 : 544.621*

## 21. Naofumi Ōi, Kazuko Kageyama, and Keiichiro Miyazaki: Studies on Intermolecular Bonds by Infrared Absorption Spectra. I. Hydrogen Bonding Power of Antipyrine and Aminopyrine.

(*Research Department, Osaka Works, Sumitomo Chemical Company**)

It is well known that antipyrine and aminopyrine form molecular addition compounds with various phenol derivatives or barbital derivatives, but the structure of their molecular compounds are not always obvious.

Hirayama, *et al.*[1] studied solvent effect on the ultraviolet absorption spectra of antipyrine and aminopyrine, and presumed that they form hydrogen bond with solvents, such as C=O····H, because their spectra show a remarkable blue shift.

However, detailed studies on how the hydrogen bond is formed between antipyrine or aminopyrine and phenol derivatives have not been made yet.

Useful data concerning this question may be obtained from infrared absorption measurements of certain ternary solutions, each of which is composed of a large quantity of nonpolar solvent (such as carbon tetrachloride) and small quantities of two polar solutes (a proton-donor containing X-H, such as phenol, and a proton-acceptor containing Y, such as antipyrine) and in which complex molecules with the hydrogen bond X-H ····Y are present.

Therefore infrared absorption spectra of various ternary solutions were measured in the region of 3000~4000 $cm^{-1}$ and 1500~2000 $cm^{-1}$. The compositions of ternary solutions examined were phenol, thymol, and 1-naphthol each with antipyrine and carbon tetrachloride, and phenol, thymol, and 1-naphthol, each with aminopyrine and carbon tetrachloride.

For comparison, infrared absorption measurements were made also of binary solutions with compositions of phenol, thymol, 1-naphthol, antipyrine, and aminopyrine, each with carbon tetrachloride.

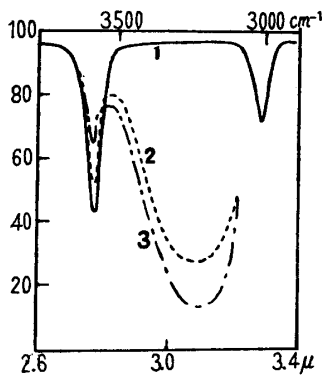
In these ternary solutions, very similar spectra were obtained and two examples of such spectra are shown in Figs. 1 and 2.

These figures show how the absorption of antipyrine or aminopyrine is affected by

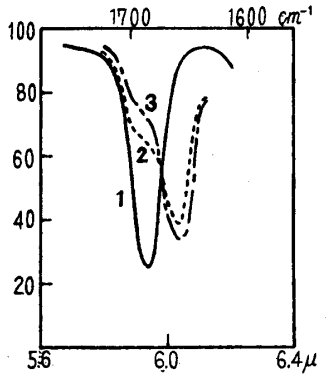
---

* Kasugade-cho, Konohana-ku, Osaka (大井尙文, 蔭山和子, 宮崎慧一郎).

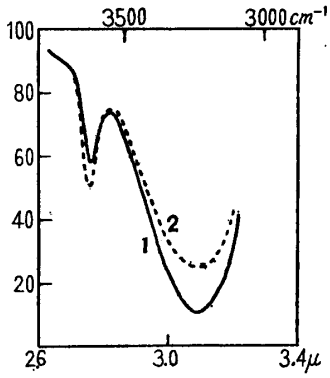
1) H. Hirayama, T. Kubota: Ann. Rept. Shionogi Research Lab., **1**, 53(1951).



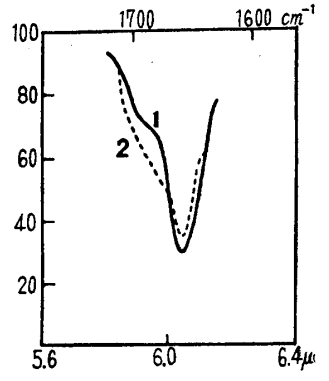

Phenol concn. 0.01 mol./L.
No. Antipyrine concn.
1 Nil
2 0.008 mol./L.
3 0.013 mol./L.
Temp. ~20° Cell, 10 mm.




Antipyrine concn., 0.011 mol./L.
No. Phenol concn.
1 Nil
2 0.021 mol./L.
3 0.043 mol./L.
Temp. ~20° Cell, 0.5 mm.




Phenol concn., 0.01 mol./L.
Antipyrine concn., 0.013 mol./L.
No. Temp.
1 ~20°C
2 ~50°C
Cell, 10 mm.




Antipyrine concn., 0.011 mol./L.
Phenol concn., 0.043 mol./L.
No. Temp.
1 ~20°C
2 ~50°C
Cell, 0.5 mm.

Fig. 1. Antipyrine + Phenol + $CCl_4$

the addition of proton-donors and how the absorption of phenol is affected by the addition of proton-acceptor to the solution.

For example, in Fig. 1, phenol has only one OH band at 3610 $cm^{-1}$ and antipyrine has only one CO band at 1689 $cm^{-1}$ in the binary solutions, but in the ternary solution two OH bands are present, at 3610 and 3215 $cm^{-1}$, and two CO bands are present at 1689 and 1662 $cm^{-1}$.

The comparison of these curves in Fig. 1 reveals that by adding antipyrine to the solution of phenol, the absorption intensity of the OH band at 3610 $cm^{-1}$ becomes weaker and there appears another OH band at 3215 $cm^{-1}$. Further experiments have shown that, on increasing the concentration of antipyrine in the solution, there occurs a decrease in the absorption intensity of 3610 $cm^{-1}$ band and an increase in that of 3215 $cm^{-1}$ band. On raising the temperature of the ternary solution, there occurs an increase in the absorption intensity of 3610 $cm^{-1}$ band and a decrease in that of 3215 $cm^{-1}$ band, but no shift occurs in the positions of these OH bands.

These facts may be interpreted as indicating the formations of an intermolecular hydrogen bond $C_6H_5OH\cdots$antipyrine in the above-mentioned ternary solution.

On the other hand, by adding phenol to the solution of antipyrine, the absorption

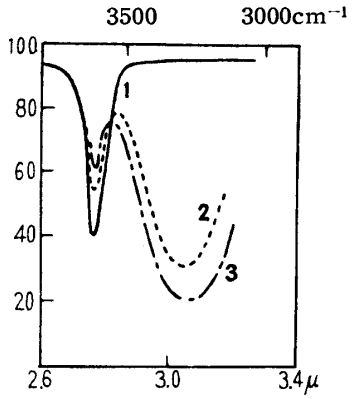
intensity of the CO band at 1689 $cm^{-1}$ becomes weaker and there appears another CO band at 1662 $cm^{-1}$. Further experiments have shown that, on increasing the concentration of phenol in the solution, there occurs a decrease in the absorption intensity of 1689 $cm^{-1}$ band and an increase in that of 1662 $cm^{-1}$ band. On raising the temperature of the ternary solution there occurs an increase in the absorption intensity of 1689 $cm^{-1}$ band and a decrease in that of 1662 $cm^{-1}$ band, but no shift occurs in the positions of these CO bands.

These facts may be interpreted as indicating the formation of an intermolecular hydrogen bond phenol$\cdots$OC($C_{10}H_{12}N_2$) in the above-mentioned ternary solutions.

It is thereby concluded that in ternary solutions, the molecular complex $C_6H_5OH\cdots$ OC($C_{10}H_{12}N_2$) is formed by intermolecular hydrogen bonding and that this complex molecule consists of free phenol and antipyrine molecules in equilibrium :

$$C_6H_5OH + OC(C_{10}H_{12}N_2) \rightleftarrows C_6H_5OH\cdots OC(C_{10}H_{12}N_2)$$

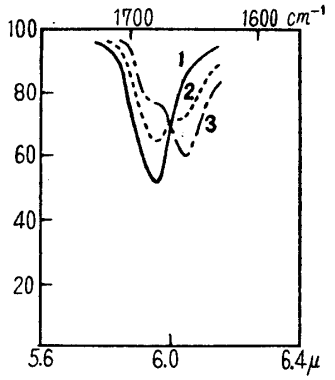
Fig. 2 shows the spectra of other ternary solutions. Each of these solutions contains, besides phenol, aminopyrine as a proton-acceptor. In these solutions, phenol exhibits OH band at 3278 $cm^{-1}$ besides that at 3610 $cm^{-1}$, and aminopyrine exhibts CO band at 1658 $cm^{-1}$ besides that at 1681 $cm^{-1}$, indicating that the complex $C_6H_5OH\cdots$OC-




Phenol concn., 0.01 mol./L.

| No. | Aminopyrine concn. |
|---|---|
| 1 | Nil |
| 2 | 0.007 mol./L. |
| 3 | 0.011 mol./L. |

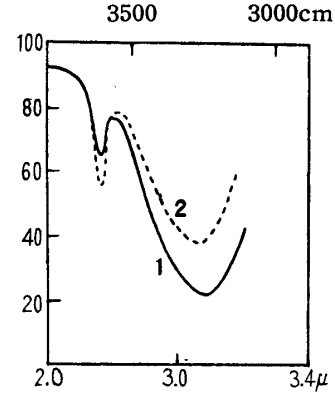
Temp. ~20° Cell, 10 mm.




Aminopyrine concn., 0.009 mol./L.

| No. | Phenol concn. |
|---|---|
| 1 | Nil |
| 2 | 0.011 mol./L. |
| 3 | 0.032 mol./L. |

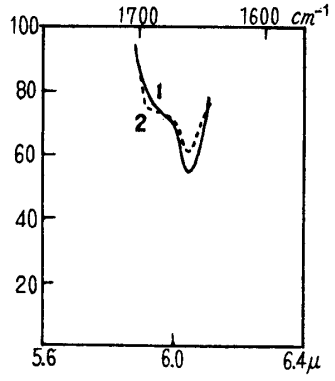
Temp. ~20° Cell, 0.5 mm.




Phenol concn., 0.01 mol./L.
Aminopyrine concn., 0.011 mol./L.

| No. | Temp. |
|---|---|
| 1 | ~20°C |
| 2 | ~50°C |

Cell, 10 mm.




Aminopyrine concn., 0.009 mol./L.
Phenol concn., 0.032 mol./L.

| No. | Temp. |
|---|---|
| 1 | ~20°C |
| 2 | ~50°C |

Cell, 0.5 mm.

Fig. 2. Aminopyrine + Phenol + $CCl_4$

($C_{12}H_{17}N_3$) is formed in the solution.

The spectra of ternary solutions in which thymol or 1-naphthol is present, instead of phenol, as a proton-donor, and antipyrine or aminopyrine is present as a proton-acceptor, are much like those in Figs. 1 and 2, and similar interpretations may be given for these data.

In these six ternary solutions the values of $\nu_{OH}$ (the wave number of hydrogen-bonded OH strectching vibration), $\Delta\nu_{OH}$ (its shift from the wave number 3610 or 3593 $cm^{-1}$ of free OH stretching vibration), and $\nu_{CO}$ (the wave number of hydrogen-bonded CO stretching vibration), and $\Delta\nu_{CO}$ (its shift from the wave number 1689 or 1681 $cm^{-1}$ of free CO stretching vibration) are given in Table I.

It is difficult to determine the exact value of $\Delta E$ (the energy of intermolecular hydrogen bond in the complex), but the order of the strengths of these hydrogen bonds in these complexes may be determined by observing the wave number of the OH band related to the hydrogen bond in question.

Generally speaking, the wave number of the hydrogen-bonded OH band is taken as a measure of the strength of the bond, if other conditions are equal, because the lower frequency of the bonded OH band indicates a greater loosening of the OH accompanying the hydrogen bond formation.

Therefore, for the comparison of the power of the hydrogen bond formation, infrared absorption measurements were made of ternary solutions of dioxane, ethyl ether, and pyridine, each with phenol and carbon tetrachloride. These data are also shown in Table I.

Table I.

| Proton donor | Proton acceptor | $\nu_{OH}$ ($cm^{-1}$) | $\Delta\nu_{OH}$ ($cm^{-1}$) | $\nu_{CO}$ ($cm^{-1}$) | $\Delta\nu_{CO}$ ($cm^{-1}$) | (Kcal./mole) $\Delta E$ | Ref. |
|---|---|---|---|---|---|---|---|
| Phenol | Antipyrine | 3215 | 395 | 1662 | 27 | 4 | Writers' |
| Thymol | 〃 | 3215 | 378 | 1662 | 27 | | |
| 1-Naphthol | 〃 | 3164 | 446 | 1658 | 31 | | |
| Phenol | Aminopyrine | 3278 | 332 | 1658 | 23 | | |
| Thymol | 〃 | 3289 | 304 | 1661 | 20 | | |
| 1-Naphthol | 〃 | 3236 | 374 | 1656 | 25 | | |
| Phenol | Dioxane | 3367 | 243 | — | — | 3.5<br>4.7<br>5.5 | a<br>b<br>c |
| 〃 | Ethyl ether | 3322 | 288 | — | — | 3.7 | a |
| 〃 | Pyridine | 3115 | 495 | — | — | 5 | a |

a) M. Tsuboi : J. Chem. Soc. Japan, **72**, 146(1951).
b) S. Nagakura : *Ibid.*, **74**, 153(1953).
c) M.C. Flett : J. Soc. Dyers Colourists, **68**, 59(1952).

Examination of Table I, with what have been just stated in mind, reveals that the OH····O=C bonds in the complex of antipyrine-phenol and aminopyrine-phenol, are stronger than the OH····O bonds in the complexes of phenol-dioxane, or phenol-ethyl ether, and weaker than the OH····N bond in the complex of phenol-pyridine.

As an example of the determination of intermolecular hydrogen bond energy, $\Delta E$ between phenol and antipyrine was obtained after Tsuboi's method[a] according to the data of the temperature change.

$$\Delta E = -\frac{T_1 T_2}{T_2 - T_1} R \ln \frac{[DA]_1/[D]_1[A]_1}{[DA]_2/[D]_2[A]_2} = -\frac{T_1 T_2}{T_2 - T_1} R \ln \frac{[DA]_1/[D]_1}{[DA]_2/[D]_2}$$

where [DA], [D], and [A] denote the concentration (mole/L.) of DA, D, and A, at two temperatures, $T_1$ and $T_2$, respectively, A is a proton acceptor, and DA is the molecular complex formed. $T_1$ and $T_2$ are absolute temperatures. The value obtained was $\Delta E = -4$ Kcal./mole.

In Table I the values of $\Delta E$ in other systems obtained by other researchers are also listed and these data reveal that the foregoing assumption on the strength of hydrogen bonding power of antipyrine may be fairly accurate, but these values are far different between researchers. It may be summarized that the hydrogen bonding power of antipyrine or aminopyrine with proton donors is considerably strong and nearly equal to that of ethyl ether, dioxane, or pyridine. It may be expected that molecular addition compounds would be formed, with strong intermolecular hydrogen bond, with compounds containing active hydrogen.

The authors wish to express their sincere gratitude to Prof. S. Takagi of the University of Kyoto for his helpful guidance, and to Messrs. K. Ōya, Y. Yamaguchi, H. Yamura, S. Yoshida, and H. Yoshida of this Company for their kind encouragements.

## Experimental

The spectra were obtained with a Perkin-Elmer Model 21 recording infrared spectrophotometer, using NaCl prism.

All of the substances studied were commercially available. These compounds were used without further purification unless doubt exsisted as to their purity, and if purification was necessary, standard methods of distillation and recrystallization were used.

## Summary

The hydrogen bonding power of antipyrine and aminopyrine was examined by the measurement of infrared absorption spectra of various ternary solutions and it was concluded that the molecular complexes $C_6H_5OH\cdots OC(C_{10}H_{12}N_2)$ and $C_6H_5OH\cdots OC(C_{12}H_{17}N_3)$ formed in the solution by intermolecular hydrogen bonding. The order of the strengths of these hydrogen bond was considerably large.

(Received January 9, 1957)

*U. D. C. 547.659.6 : 544.621*

## 22. Naofumi Ōi, Kazuko Kageyama, and Keiichiro Miyazaki : Studies on Intermolecular Bonds by Infrared Absorption Spectra. II.[1] Hydrogen Bonding Power of Santonin.

(*Research Department, Osaka Works, Sumitomo Chemical Co.**)

Kakemi, *et al.*[2] studied molecular addition compounds of santonin with various resorcinol derivatives by thermoanalyses, but the structure of the molecular compounds was not examined. In this paper the hydrogen bonding power of santonin is presented for studying its intermolecular bonds.

In the previous paper,[1] we reported the hydrogen bonding power of antipyrine and aminopyrine by the infrared absorption spectra, and the same method was used in this study.

The infrared absorption spectra were measured in the region of 3000～4000 $cm^{-1}$ and 1500～2000 $cm^{-1}$ in three ternary solutions.

The compositions of the ternary solutions examined were phenol, thymol, and 1-naphthol, each with santonin and carbon tetrachloride.

* Kasugade-cho, Konohana-ku, Osaka (大井尙文, 蔭山和子, 宮崎慧一郎).

1) Part I : This Bulletin, **5**, 141(1957).

2) K. Kakemi, T. Uno, Y. Sanada : Archive of Practical Pharmacy, **12**, 52(1953).