# Molecular Conformations of γ-Aminobutyric Acid and γ-Amino-β-hydroxybutyric Acid in Aqueous Solution†

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Proton magnetic resonance and Raman spectra indicate that the backbone of γ-aminobutyric acid is fairly flexible and rotational isomers are almost equally populated in aqueous solution. The population is not much affected by change of pH. On the other hand, in γ-amino-β-hydroxybutyric acid the *trans*†† -*trans* and *gauche-trans* conformations of the backbone are equally populated in neutral solution, *trans-trans* conformation being predominant in non-zwitterionic forms. The conformation in solution is discussed in relation to the structure of these molecules in crystal.

γ-Aminobutyric acid (GABA) has been found in mammalian brain,[1] where it is enzymatically produced from glutamic acid.[2] By virtue of its potent synaptic effect and other evidences, GABA seems to play the physiological role as "inhibitory transmitter" in mammalian central nervous system.[3–6] γ-Amino-β-hydroxybutyric acid (GABOB) is also a central inhibitory transmitter like GABA.[7] Their chemical structures are shown in Fig. 1. The crystal structures of GABA, GABOB, and their analogues have been reported.[8–13]

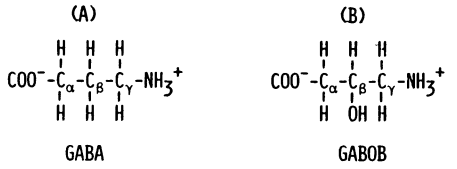


Fig. 1. Chemical structures of (A) γ-aminobutyric acid (GABA) and (B) γ-amino-β-hydroxybutyric acid (GABOB).

It is remarkable that a difference in the conformation of GABA molecule was found for crystal structures of GABA[9] and its hydrochloride:[10] the conformation of $C_\alpha$–$C_\beta$–$C_\gamma$–N skeleton of GABA hydrochloride is all *trans*, but that of GABA in its free form is *gauche* (73.6°) about the $C_\alpha$–$C_\beta$ bond and *trans* about the $C_\beta$–$C_\gamma$ bond, while GABOB takes the all *trans* conformation as GABA hydrochloride.[11]

The preferred conformation of GABA is theoretically predicted by molecular orbital calculations.[14–16] Pullman and Berthod[16] predicted that the intrinsically preferred conformation of the isolated GABA molecule is a highly holded one, but the hydrated zwitterionic GABA molecule might exist as a mixture of a number of conformers having different degrees of folding.

In the present study, we aimed to elucidate the molecular conformations of GABA and GABOB in aqueous solution by means of proton magnetic resonance (PMR), attempting to correlate the conformations in solution with those in crystal by measuring laser Raman spectra.

† This paper is Part VIII of "Crystal and Molecular Structure of ω-Amino Acids, ω-Amino Sulfonic Acids, and Their Derivatives."

†† The term *trans* used in the present paper denoted *s-trans* or *anti* in the nomenclature of stereochemistry.

## Materials and Procedures

GABA and racemic GABOB (Nakarai Chemical Co., Kyoto) were used without further purification. GABA hydrochloride was prepared from GABA and crystallized.

Proton magnetic resonance spectra were recorded on a JEOL-PFT-100 pulse Fourier transform NMR spectrometer equipped with temperature control apparatus. The probe temperature was kept at 45 °C unless otherwise stated. The resolution due to digitalization by computer is 0.04 Hz. The sample solutions were prepared in $D_2O$ with concentration of 100 mg/ml. The pD value was adjusted with DCl and NaOD. Sodium 3-(trimethylsilyl)propionate-2,2,3,3-$d_4$ (TMSP-$d_4$) was used as an internal reference. The assignments of resonance signal were made by comparison with those of GABA and GABOB analogues, 3-aminopropanesulfonic acid, 3-amino-2-hydroxypropanesulfonic acid and γ-guanidinobutyric acid. The spin analysis of the spectra was accomplished by computer with an iterative program based on the least-squares method. The spectra were analyzed as six spin system for GABA and five spin system for GABOB. Raman spectra were taken with a JEOL-JRS-02AS and JEOL-JRS-400D Raman spectrometer by excitation with argon ion laser (wavelength, 488.0 nm).

## Results and Discussion

*PMR Spectra of γ-Amino-β-hydroxybutyric Acid (GABOB).* The 100-MHz proton magnetic resonance spectra of GABOB in solution at pD 1.7 and 7.2 are shown in

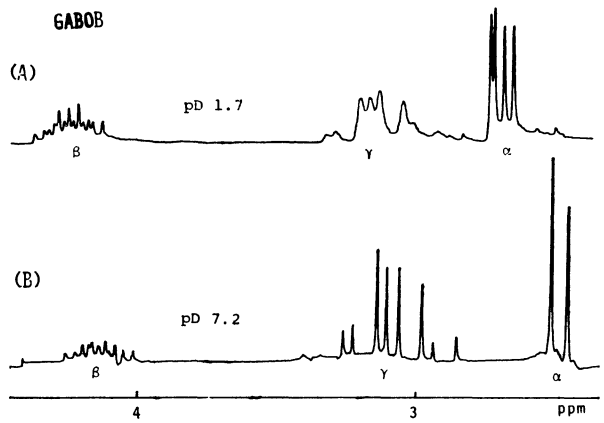


Fig. 2. The 100 MHz PMR spectra of γ-amino-β-hydroxybutyric acid in $D_2O$ (A) at pD 1.7 and (B) at pD 7.2. Assignment are shown in the spectra.

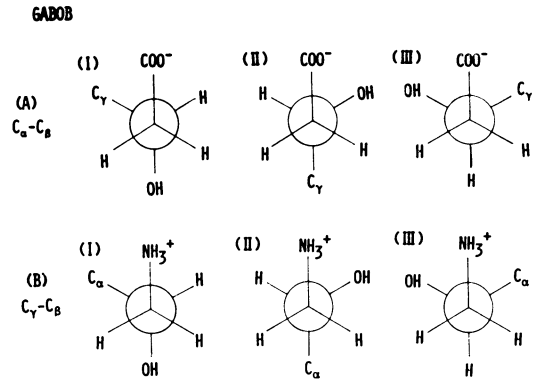


Fig. 3. The possible rotational isomers of γ-amino-β-hydroxybutyric acid (A) about the $C_\alpha$–$C_\beta$ bond and (B) about the $C_\beta$–$C_\gamma$ bond.

Fig. 2. GABOB is considered to exist as a mixture of three possible rotational isomers both about the $C_\alpha$–$C_\beta$ bond and about the $C_\beta$–$C_\gamma$ bond. The Newman projections of the possible rotamers of zwitterionic GABOB molecule which has the ABX spin system are shown in Fig. 3. The rotamers are thought to interconvert rapidly to each other. The observed coupling constants are weighted mean values of those for the distinct rotamers.

The vicinal coupling constants are assumed to satisfy the Karplus equation[17] and the coupling constants of either *gauche* or *trans* conformer are assumed to have the same value regardless of the particular conformation. The vicinal coupling constants, $J_{ax}$ and $J_{bx}$, are then given by

$$J_{ax} = P_I J_t + P_{II} J_g + P_{III} J_g, \quad (1)$$

$$J_{bx} = P_I J_g + P_{II} J_t + P_{III} J_g, \quad (2)$$

where $P_I$, $P_{II}$, and $P_{III}$ are the populations of the rotamers I, II, and III, respectively, and $J_g$ and $J_t$ are the coupling constants for the *gauche* and *trans* conformations with respect to the two C–H bonds.

In this case, $J_g$ was determined by application of the Karplus type equation to hydroxyproline,[18] since the $C_\alpha$–$C_\beta$ part of GABOB corresponds to the $C_3$–$C_4$ part of hydroxyproline and the $C_\beta$–$C_\gamma$ of GABOB to the $C_4$–$C_5$ of hydroxyproline. Thus $J_g$ of GABOB was assumed to be 2.73 Hz with respect to the $C_\alpha$–$C_\beta$ and 2.53 Hz for the $C_\beta$–$C_\gamma$ bond, respectively. $J_t$ was determined as follows according to the method reported by Abraham and Pachler.[19] The average coupling constant is defined by

$$J_{av} = 1/3(J_t + 2J_g). \quad (3)$$

On the other hand, $J_{av}$ was found to be proportional to the total electronegativities as follows.

$$J_{av} = 17.97 - 0.796 \sum_{n=1}^{6} E_n \quad (4)$$

With use of Huggins' electronegativities,[20] $J_t$ was calculated to be 11.92 Hz for $C_\alpha$–$C_\beta$ part and 11.24 Hz for the $C_\beta$–$C_\gamma$ part.

Figure 4 shows the populations of GABOB conformers calculated from the coupling constants obtained at various pD values. In the case of GABOB, it is impossible to assign $J_{ax}$ and $J_{bx}$, namely, $P_I$ and $P_{II}$ only

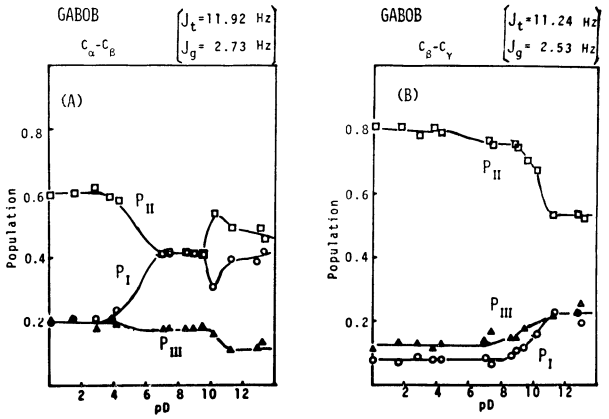


Fig. 4. The effect of pD on the populations of γ-amino-β-hydroxybutyric acid (A) about the $C_\alpha$–$C_\beta$ bond and (B) about the $C_\beta$–$C_\gamma$ bond.

from the spin analyses. The rotamer populations mainly depend on steric and ionic interactions. It seems that rotamer II is more favorable and more stable than rotamers I and III for non-zwitterionic structure as considered from the attractive interactions between the hydroxyl and the polar groups. Thus we assigned the predominant conformer to rotamer II. The assumption is supported by the unambiguously determined population for some β-monodeuterated amino acid.[21] Thus about the $C_\alpha$–$C_\beta$ bond in the zwitterionic form, rotamers I and II equally exist with a population of about 40% as shown in Fig. 4 mainly due to the ionic interactions of the ionized amino and carboxyl groups. The effect of intermolecular interaction on population can be neglected since the vicinal coupling constants do not change in any of the concentrations 10, 1, and 0.1%.

It appears anomalous with increase in pD from neutral to alkaline, that the vicinal coupling constants of rotamers I and II about the $C_\alpha$–$C_\beta$ bond once diverge and converge again to a common value; the vicinal coupling constants diverge at pD 10 by deprotonation of the amino group whose $pK_a$ is about 9.5 obtained from the PMR chemical shift. However, it seems impossible to explain the convergence of the constants at about pD 10 only by the change in intramolecular interactions. Figure 5 shows the temperature dependence of the coupling constants of the GABOB molecule

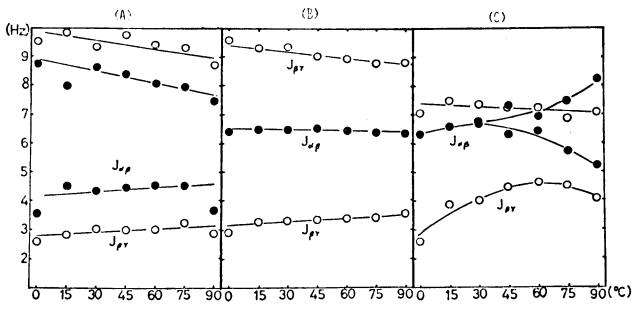


Fig. 5. The observed vicinal coupling constants at various temperatures for γ-amino-β-hydroxybutyric acid: (A) cationic form, (B) zwitterionic form and (C) anionic form.

at anionic, zwitterionic and cationic states, respectively. It is intuitively expected that when the temperature increases, the rotamer populations would tend to become equal according to the Boltzmann distribution; the coupling constants, therefore, would converge to a common value. At either zwitterionic or cationic state, the constants actually converged with increasing temperature, but they diverged at anionic state in alkaline solution. Another effect of intramolecular interaction accessible only in the anionic state should be considered. From the NMR studies of $^{23}Na$ ion in aqueous solution,[22] it was suggested that the sodium ion forms weak complex with several anions, notably with hydroxy and keto acids such as $\alpha$- and $\beta$-hydroxybutyric acid.

As regards the $C_\beta$–$C_\gamma$ bond, rotamer II is the most stable at any pD value. This is similar to the case of acetylcholine[23] and phosphatidyl choline and phosphatidyl ethanolamine[24] in which the O–C–C–N system is in the *gauche* conformation in aqueous solution. If there is no correlation between the population about the $C_\alpha$–$C_\beta$ bond and that about the $C_\beta$–$C_\gamma$ bond in neutral solution, about 30% of GABOB molecules have the *trans*(about the $C_\alpha$–$C_\beta$ bond)-*trans*(about the $C_\beta$–$C_\gamma$ bond) conformation as well as the *gauche-trans* conformation. Thus the *trans-trans* conformation of GABOB found in the crystal structure may correspond to one of the stable conformations in aqueous solution as anticipated from the PMR studies. In acidic solution, on the other hand, about 50% of GABOB molecules are in the *trans-trans* conformation and about 15% of GABOB in the *gauche-trans*.

*PMR Spectra of γ-Aminobutyric Acid (GABA).* Figure 6 shows the 100 MHz proton magnetic resonance spectra of GABA at pD 13.3, where the upper is the magnified spectrum of lower one. GABA is considered to exist as a mixture of three rotational isomers in regard to both the $C_\alpha$–$C_\beta$ bond and the $C_\beta$–$C_\gamma$ bond. The Newman projections of the rotamers of the zwitterionic GABA molecule are shown in Fig. 7. In this case, it was considered that GABA has the $A_2B_2$ spin system both about the $C_\alpha$–$C_\beta$ and the $C_\beta$–$C_\gamma$ bonds. The $A_2B_2$ type spectra are described in terms of four coupling constants, $J$, $J'$, $J_t$, and $J_g$, defined by

$$J = P_t J_g + 1/2 P_g (J_t + J_g), \quad (5)$$

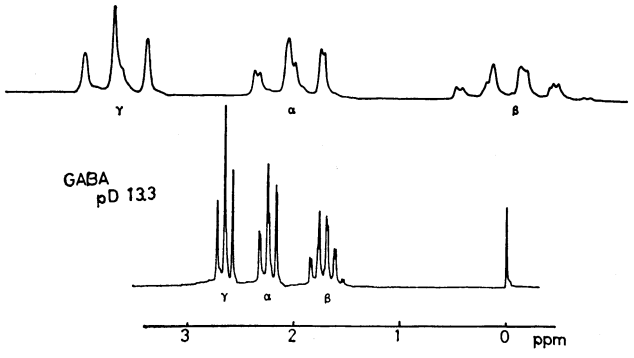


Fig. 6. The 100 MHz PMR spectra of γ-aminobutyric acid in $D_2O$ at pD 13.3. The upper is the magnified spectrum of the lower one in the region between 1.4 and 2.8 ppm.

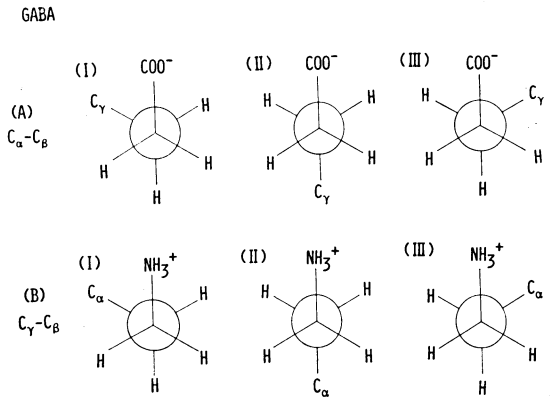


Fig. 7. The possible rotational isomers of γ-aminobutyric acid (A) about the $C_\alpha$–$C_\beta$ bond and (B) about the $C_\beta$–$C_\gamma$ bond.

$$J' = P_t J_t + P_g J_g, \quad (6)$$

where $P_t$ and $P_g$ are the populations of the *trans* and *gauche* rotamers, respectively, and

$$L = J - J' = (1/2 - 3/2P_t)(J_t - J_g), \quad (7)$$

$$P_t = 1/3 - 2L/3(J_t - J_g). \quad (8)$$

Eq. 8 indicates that if $L$ is positive, $P_t$ is smaller than 1/3 and; if negative, $P_t$ is larger than 1/3, since $(J_t - J_g)$ is positive in general. In this analysis, the sign of $L$, namely, $J$ and $J'$, could not be assigned to each of the coupling constants obtained from the spin analysis. The average coupling constant is defined by

$$J_{av} = 1/3(2J + J'). \quad (9)$$

As regards Eq. 4 Abraham and Pachler[19] found the relation between the average coupling constants $J_{av}$ and the electronegativity of attached atoms. In the case of pD 13.3, the $3J_{av}$ value about the $C_\alpha$–$C_\beta$ bond is 22.9 Hz by virtue of Eq. 9, if $L$ is assumed to be positive, and $3J_{av}$ becomes 21.8 Hz if $L$ is negative, while Eq. 4 gives 20.49 Hz for $3J_{av}$. By comparison of the three values, it seems likely that the sign of $L$ is negative. Likewise, if $L$ is positive, $3J_{av}$ about the $C_\beta$–$C_\gamma$ bond should be 22.3 Hz. If $L$ is negative, it becomes 21.2 Hz, whereas it is calculated to be 19.41 Hz from Eq. 4. Thus the sign of $L$ is also considered to be negative.

The value obtained for $L$ and calculated populations of the rotamers from the spin analyses at pD 13.3 and 7.6 are given in Table 1. The coupling constant at pD 0.9 could not be obtained, because of the broadening of the $C_\gamma$-proton signals. Only the data at pD 7.6 about the $C_\alpha$–$C_\beta$ bond are given in the Table, since $L$ for $C_\beta$–$C_\gamma$ is too small (<0.2 Hz) to be determined

Table 1. Populations of *trans* and *gauche* forms in GABA

| Torsional axis | pD | $J$(Hz) | $J'$(Hz) | $L$(Hz) | $P_t$ | $P_g$ |
|---|---|---|---|---|---|---|
| $C_\alpha$–$C_\beta$ | 13.3 | 6.9 | 8.0 | −1.1 | 0.40 | 0.60 |
| | 7.6 | 7.0 | 7.6 | −0.6 | 0.37 | 0.63 |
| $C_\beta$–$C_\gamma$ | 13.3 | 6.7 | 7.8 | −1.1 | 0.40 | 0.60 |

by the least squares method.

It is of interest to compare the conformation of the GABA molecule in solution at various pD with the crystal structure and the predicted conformations from theoretical calculations. Five possible conformations of GABA molecule are *trans-trans gauche-trans*, *trans-gauche*, *gauche-gauche*(A) and *gauche-gauche*(B) as the rotational isomers about the $C_\alpha$–$C_\beta$ bond and the $C_\beta$–$C_\gamma$ bond. If the population of trans isomer is 1/3 about both the $C_\alpha$–$C_\beta$ bond and the $C_\beta$–$C_\gamma$ bond, the estimated populations of the five conformations are 1/9, 2/9, 2/9, 2/9, and 2/9, respectively. As the result of PMR studies of GABA, the population of each isomer in alkaline solution is 0.16(*trans-trans*), 0.24(*gauche-trans*), 0.24(*trans-gauche*), 0.18(*gauche-gauche*(A)), and 0.18 (*gauche-gauche*(B)). The *trans-trans* conformation is a little more predominant than the case of freely rotating molecule. Although the spectrum of GABA in acidic solution is too broad to be used for spin analysis, its pattern does not differ a great deal from that of alkaline solution. Thus the conformation of GABA in acidic solution might be similar to that in alkaline solution. The crystal structure of GABA hydrochloride shows the GABA molecule to be *trans-trans*, while the molecular conformation of neutral GABA in the crystal is *gauche-trans*. The present PMR data and the crystal data both imply that the energy difference between the two conformers is small. The GABA molecule in aqueous solution can take many conformations, and the change in population by pH is estimated within 10%. The extended Hückel MO calculation predicted that the *trans-trans* conformation is most stable for the zwitterionic GABA molecule,[14] not agreeing with our PMR result. The CNDO/2 calculations also predicted that for non-zwitterionic form six stable conformations involving all *trans* and for zwitterionic form some folded conformations are preferable.[15] The result is not in line with ours. The PCILO and SCF *ab initio* calculations by Pullman and Berthod[16] showing GABA to be a mixture of a number of conformers agree with our PMR result.

*Raman Spectra of γ-Aminobutyric Acid and γ-Amino-β-hydroxybutyric Acid.* The Raman spectra of GABOB in solid state, neutral solution and acidic solution are given in Fig. 8. Because of the complex spectra, it is hard to assign each band to the definite vibrational mode. From X-ray diffraction studies, the conformation of GABOB was found to be *trans-trans* in the solid state. From PMR studies the *trans-trans* conformation is thought to exist *ca.* 30% in neutral solution and *ca.* 50% in acidic solution. As a whole the Raman spectrum in solid and that in neutral solution is similar to each other. But no Raman line corresponding to the line at 830 $cm^{-1}$ either in neutral or in acidic solution was observed in solid. Therefore, the Raman line at 830 $cm^{-1}$ is thought to be due to the *gauche-trans* conformation, differing from the *trans-trans* conformation found in crystal.

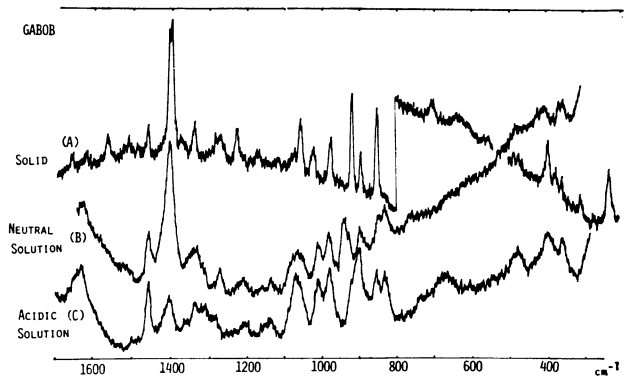


Fig. 8. The Raman spectra of γ-amino-β-hydroxybutyric acid (A) in solid, (B) in neutral solution and (C) in acidic solution.

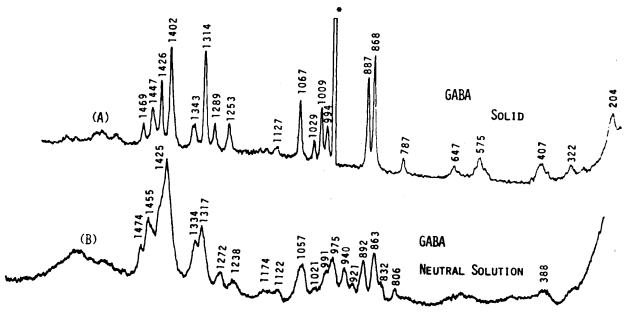


Fig. 9. The Raman spectra of γ-aminobutyric acid (A) in solid and (B) in neutral solution. The Raman line with asterisk is due to the ghost of spectrometer.

The Raman spectra of GABA in solid state and in neutral solution are given in Fig. 9. The spectral features differ from each other: the Raman lines are observed at 921 and 940 $cm^{-1}$ in neutral solution, but there is no line in the range 887—994 $cm^{-1}$ in solid state. These lines which are sensitive to the conformational change should be mainly due to the stretching modes of the C–C skeletal bonds or the rocking modes of the $CH_2$ group. The Raman spectra of GABA hydrochloride either in solid or solution are also not very similar to each other, especially in the region 800—1100 $cm^{-1}$. It is apparent that the molecular conformation of GABA found in crystal is not retained in solution.

## Conclusion

Proton magnetic resonance and Raman spectra indicate that the backbone of the GABA molecules is fairly flexible and probable rotational isomers are almost equally populated in aqueous solution. The population is not much affected by changing pH. On the other hand, the GABOB molecule tends to take *trans* conformation about each backbone bond. The *trans-trans* and *gauche-trans* are equally populated in neutral solution, the *trans-trans* conformation being predominant in the non-zwitterionic forms. In the crystal, zwitterionic GABA molecule is in the *gauche-trans* form and its hydrochloride takes the *trans-trans* form, while the neutral GABOB is in the *trans-trans* form. It is interesting to note that the molecular structure of GABA in the crystal is restricted to the *trans-trans* and *gauche-trans* forms, and the GABOB molecules take mainly the *trans-*

*trans* and *gauche-trans* conformations in solution. It seems that GABA and GABOB are apt to take either of the two forms under certain circumstances, the forms being important for the exhibition of their biological activities.

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