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Tokunosuke Kanzawa and Toru Masuda : A Note on the Infrared Spectra of Deuterated Riboflavin.

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The spectral changes caused by deuteration of a compound can give valuable informations on the assignment of infrared adsorption bands and accordingly on the structure of the compound.[1] In order to obtain some informations useful for determining the structure of unknown substances obtained by Masuda[2] from the mycelium and broth of the culture of *Eremothecium ashbyii*, infrared spectra of partially deuterated riboflavin and related compounds were recorded.

Generally, the hydrogen atom of OH or NH group is readily substituted by deuterium when the compounds having such groups are recrystallized from deuterium oxide. It is expected that by the deuteration an absorption band due to OH and NH stretching or deformation vibration shifts towards a lower frequency. As shown in Fig. 1, absorption bands in 3300 cm^{-1} region become weak on deuteration and new bands appear in 2500 cm^{-1}. These bands originate from the OH and NH stretching vibrations and their complexity may be due to the existence of hydrogen bonds of different strength formed by many OH groups of the carbohydrate and NH groups in the pteridine ring. The ratios of the wave numbers of the bands near 3300 cm^{-1} to those of the bands near 2500 cm^{-1} amount to about 1.35. Lumazine and alloxan which have no ribityl group also show a new band near 2500 cm^{-1} probably due to the NH group of lactam form in the pteridine ring. It seems that the absorption in this region of compounds having ribityl group is more complicated than that of the ones having no ribityl group.

The changes of absorptions near 1600 cm^{-1} on deuteration cannot be easily explained. For example, for adenosine it will be expected that the band due to NH_2 deformation vibration near 1600 cm^{-1} will shift towads lower frequency region on deuteration. However, as shown in Fig. 1-b, a new band appears at 1629 cm^{-1} close by 1669 cm^{-1} band which disappears on deuteration. Such a fact may suggest that each band does not arise from purely independent origin. Recent investigations on amides[3] have revealed that the amide-I band (1650$\sim$1600 cm^{-1}) is mainly related to $\nu_{C=O}$ with contribution of ν_{C-N} and the amide-II band (1550$\sim$1500 cm^{-1}) is related to ν_{C-N} with considerable contribution of ν_S and δ_{N-H}. The compounds a, e, and f in Fig. 1 have oxopteridine or hydroxypyrimidine rings and can be regarded as existing in lactam form or in a kind of cyclic amide form. However, the spectral change of these compounds on deuteration is not similar to that of amides. This may be caused by complicated tautomerism of the dihydroxypteridine rings as in the case of uracil.[4]

Although carbohydrates have a complicated absorption due to their OH groups in a 1000$\sim$1100 cm^{-1} region,[1] their spectral change on deuteration has not been published. It has been reported that absorption at 1010$\sim$1030 cm^{-1} due to primary alcohol shifted to 915$\sim$947 cm^{-1} on deuteration.[5] As seen in Fig. 1 the compounds having ribityl group show a marked change in the range of 1000$\sim$1100 cm^{-1} by deuteration. Such a change

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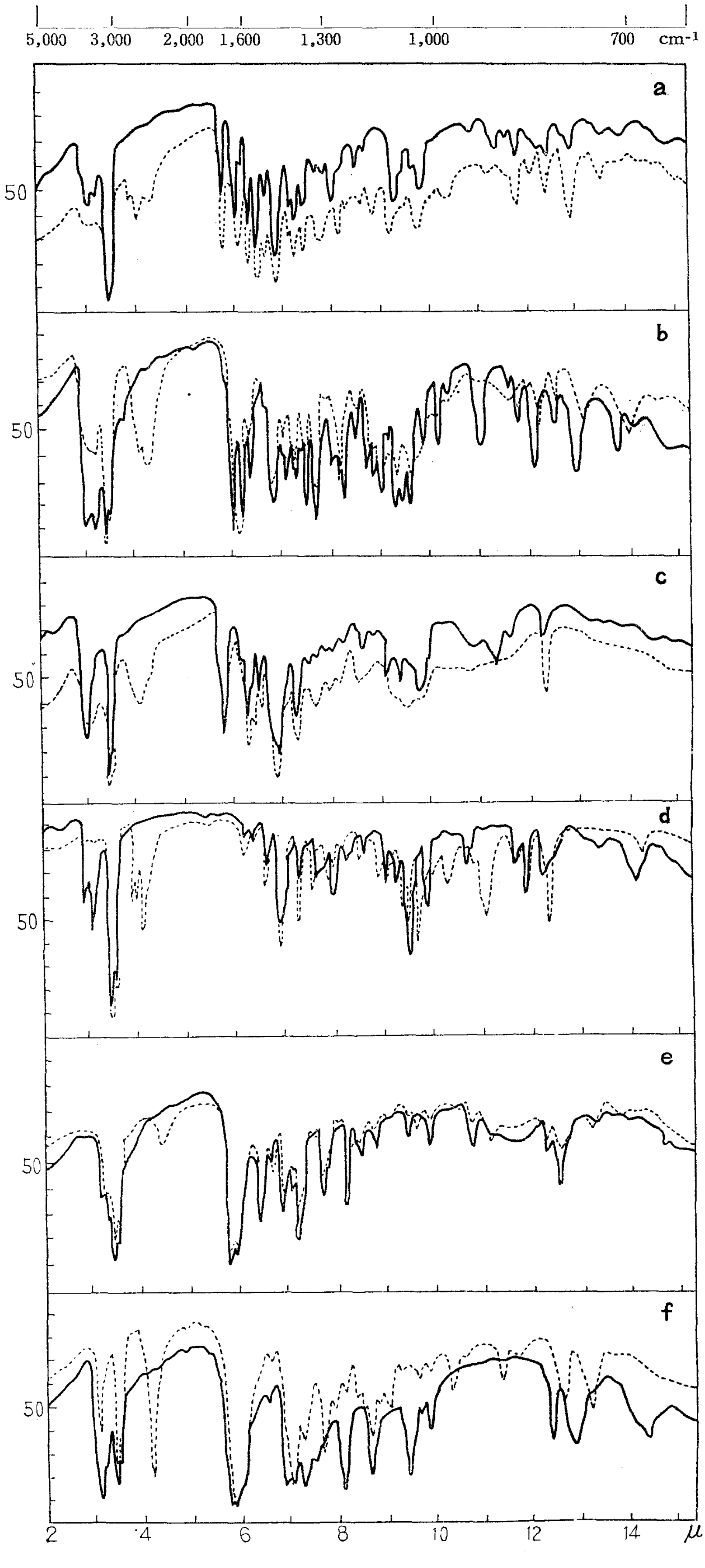
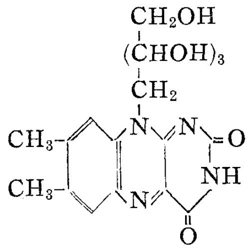
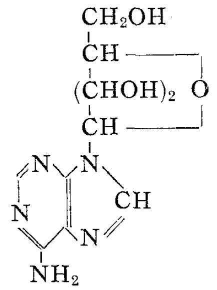
Fig. 1.

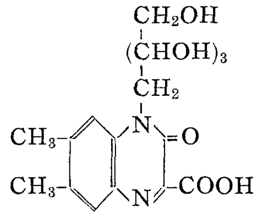




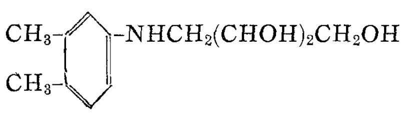
a) Riboflavin



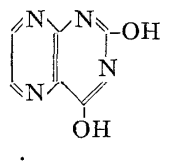
b) Adenosine



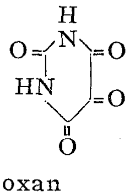
c) 6,7-Dimethyl-2-oxo-1-(1′-D-ribityl)quinoxaline-3-carboxylic acid



d) 1,2-Dimethyl-4-(1′-D-ribityl)-amino benzene



e) Lumazine



f) Alloxan

------ deuterated compounds

will be made much clear by more sufficient deuteration.

There is another band near 1250 cm^{-1} (Fig. 1) which shows marked decrease of intensity by deuteration. This band may be related to oxo-pteridine ring, although it cannot be determined whether it is due to OH group of lactim forms as explained by Short *et al.*[4] or due to NH group of lactam form as in the case of amide (amide-Ⅲ band).

From these observations it may be said with considerable certainty that the existence of a ribityl group in the compound examined is confirmed by the spectral change on deuteration in 3300 and 1000~1100 cm^{-1} region. It may be added to the above result that an absorption at 2257 cm^{-1} of lumazine gives the lowest value near 2500 cm^{-1} compared to the absorption of the other compounds and may originate from the formation of ring dimer by hydrogen bonding as found for hydroxypyrimidines.[6]

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Experimental

Deuterium oxide (Norsk Hydro) is of 99.8% in purity. Riboflavin and lumazine were recrystallized in 1:1 mixture of deuterium oxide and dioxane and other compounds were recrystallized in deuterium oxide. The spectra were taken in Nujol mull by Perkin-Elmer Model 21 Spectrophotometer with NaCl prism.

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