CXI. THE ABSORPTION SPECTRA OF SOME INDOLE DERIVATIVES.

BY FRED WILBERT WARD.

From the Biochemical Laboratory, Cambridge.

(Received August 8th, 1923.)

IN the course of an investigation on the metabolism of tryptophan and some of its derivatives, it was thought advisable to study the absorption spectra of several of these compounds.

Apparatus.

The apparatus used for the measurement of these absorption spectra was a quartz ultra-violet spectra photometer made by Adam Hilger, Ltd. It consisted of a quartz ultra-violet spectragraph with an electrode holder, a 1/4 kilowatt transformer, a suitable condenser and a rotating sector photometer. The spectragraph took a plate 10×4 inches and also contained a wave length scale mounted inside the spectragraph. By means of this scale the wave length could be photographed on the plates.

For electrodes, nickel rods were found to be the best for this kind of work. The emission spectrum of nickel is nearly as rich in lines as that given by iron and continues as far down as 2200 A.U. whereas the spectrum of iron has relatively few lines below 2500 A.U. Copper gives an emission spectrum which goes as far down as 2100 A.U. but the lines are not nearly as plentiful and it does not give nearly as satisfactory results in the measurement of absorption spectra.

Method.

In the Adam Hilger spectra photometer, simultaneous adjacent pairs of photographs of the spark across the electrodes are taken, one of which is through a cell containing the solution of the material to be measured and the other through a cell containing the solvent. The photograph through the solvent is to be considered the standard with which the other is to be compared. A number of such pairs of photographs are taken and at the finish of each exposure the sector wheel in front of the solvent or standard is closed down, the amount of light allowed to pass through by the sector wheel unto the photographic plate being in a definite ratio to the light that would pass through the solution providing no absorption were taking place. After the plate has been completely exposed, developed, washed and dried, the point of equal blackening is marked on each of the simultaneously taken adjacent pairs of photographs.

The absorption curves are plotted on cross section paper in which one ruling is arithmetic and the other logarithmic. The wave length is plotted on the arithmetic scale and the molecular absorption coefficient on the logarithmic scale. The molecular absorption coefficient is calculated from the following equation.

$$M = \frac{1}{d} \times \frac{\log I_0 - \log I}{C}.$$

The Adam Hilger sector photometer is graduated to read in terms of

$$\log I_0 - \log I.$$

Hence the readings on the sector wheel may be called α and the equation then becomes

$$M = \frac{1}{d} \times \frac{\alpha}{C},$$

where M is the molecular absorption coefficient,
 d is the thickness of the absorbing layer in centimetres,
 $\log I_0$ is the logarithm of the wave-length of the incident light,
 $\log I$ is the logarithm of the wave-length of the transmitted light,
 and C is the molecular concentration.

Experimental.

In all ten different compounds were measured. These ten compounds form four groups.

Group I: Indole, β -indolecarboxylic acid, and β -indole-aldehyde.

The indole was obtained from one of the drug houses. It was carefully purified by boiling with animal charcoal and recrystallising from dilute alcohol.

The β -indolecarboxylic acid was prepared by means of the Grignard reaction [Majina and Kotake, 1922]. It was recrystallised by dissolving in acetone and pouring into water [Ellinger, 1906]. This gave a white crystalline solid melting at 213°.

The β -indole-aldehyde was prepared by oxidising tryptophan with ferric chloride [Hopkins and Cole, 1903; Ellinger, 1906]. It was boiled with animal charcoal and recrystallised from dilute alcohol.

Fig. 1 shows the absorption curves of these compounds.

Group II: β -Indole-ethyl alcohol, β -indolepropionic acid and β -indole-aminopropionic acid or tryptophan.

The β -indole-ethyl alcohol was prepared from tryptophan by the action of yeast fermentation [Felix Ehrlich, 1922]. It was decolorised by boiling with animal charcoal and carefully recrystallised from very dilute alcohol.

The β -indolepropionic acid was prepared from tryptophan by the action of anaerobic bacteria under anaerobic conditions. The bacteria used were a mixture of *B. coli*, *B. chauvei*, *B. sporogenes* and *B. oedemaciens* [Nencki, 1889; Hopkins and Cole, 1903]. The introduction of the four different strains is a modification of the previous methods and will be published in more detail elsewhere. The indolepropionic acid was carefully decolorised with animal charcoal and recrystallised from dilute alcohol.

The tryptophan was prepared by the original method of Hopkins and Cole [1901]. It was very carefully decolorised with animal charcoal and recrystallised from 65 % alcohol.

Fig. 1.

Fig. 2.

Fig. 2 gives the absorption curves of these compounds.

Group III: α - β -Dihydroxyindole and α -hydroxy- β -indole-aldehyde.

The α - β -dihydroxyindole was prepared from isatin by reducing with sodium hydrosulphite [Marschalk, 1912]. It was carefully decolorised with animal charcoal and recrystallised from water.

The α -hydroxy- β -indole-aldehyde was prepared from thio-indigo Scharlach, R. Kalle and Co. [Friedländer and St Kielbasinski, 1912]. It was boiled with animal charcoal and recrystallised from dilute alcohol. Pale yellow crystals were obtained melting at 208° .

Fig. 3 shows the absorption curves of these compounds.

Group IV: Isatin and sodium indigosulphonate.

The isatin was obtained from a drug house and carefully boiled with animal charcoal. It was then recrystallised from dilute alcohol.

The sodium indigosulphonate or indigo carmine was a sample of Kahlbaum's pure chemicals and was used without further purification.

Fig. 4 shows the absorption spectra curves.

Discussion.

In examining the charts of group I, namely indole, β -indolecarboxylic acid and β -indole-aldehyde, one may observe the effect of substituting carboxyl and aldehyde groups in the β -position of the indole ring. Indole has a band with the tip at 2700 A.U. and with a molecular absorption coefficient of about 9000. The effect of substituting a carboxyl group in the β -position is to shift the band about 100 A.U. towards the red end of the spectrum but the intensity of the absorption is not changed. The effect of substituting an aldehyde group in the β -position is different. The band is also shifted towards the red end of the spectrum but the shift is much greater, being about 400 A.U. or four times that due to the carboxyl group. The intensity of the absorption is also increased from a molecular absorption coefficient of 9000 to 15,000.

V. Henri [1912] has shown that the carboxyl group of the aliphatic acids, such as acetic and propionic acid, absorbs the rays in the region of 2200 to 2400 A.U., the intensity increasing and shifting towards the red with increasing molecular weight of the substance. This same type of absorption may be observed in β -indolecarboxylic acid. Henri has also shown that aliphatic aldehydes such as acetaldehyde and propylaldehyde have a characteristic type of absorption curve. These curves rise to a peak around 2600 A.U., fall off again and then rise again in the extreme ultra-violet around 2200 A.U. This same type of curve is found in the lower end of the absorption spectrum of β -indole-aldehyde. Fig. 1 c shows this type of curve.

In examining the charts of group II, which consists of β -indole-ethyl alcohol, β -indolepropionic acid and β -indole-aminopropionic acid, it will be observed that the tips of the bands of all three are at about the same molecular absorption coefficient. It should be noted that these three compounds are substituted compounds of β -ethylindole, the substitution being in the side chain and being firstly a hydroxyl group, secondly a carboxyl group and thirdly an amino and a carboxyl group.

The point to be noted here is that the substitution in this side chain has very little effect in shifting the band towards the red end of the spectrum, although it has been shown that substitution in the ring by a carboxyl group shifts the band 100 A.U. in this sense. The alteration in the absorption follows the course which it would take if the compounds were entirely aliphatic. Henri has shown that the hydroxyl groups of aliphatic alcohols have an absorption band in the region of 2100 to 2200 A.U. This absorption band is

less intense than that given by corresponding aliphatic acid carboxyl groups. It is also further towards the extreme end of the ultra-violet. A similar relation holds between the lower parts of the curves of β -indole-ethyl alcohol and β -indolepropionic acid as can be seen in Fig. 1*a* and 1*b*.

In the case of β -indole-aminopropionic acid there is both an acidic and a basic group, giving an opportunity for salt formation. Salt formation has been shown by Henri [1913] to shift the absorption band into the extreme end of the ultra-violet. Salt formation appears to have taken place in this case, since that part of the absorption spectrum corresponding to the amino and carboxyl groups is further in the ultra-violet than the corresponding part for β -indolepropionic acid. The intensity is also less.

The substitution of the side chain in the indole ring acts in a similar manner to the substitution of a methyl group and shifts the absorption band about 50 A.U. towards the red. But the intensity of the absorption is decreased from 9000 to 5000 units.

In examining group III it is seen that the two members are α - and β -disubstituted compounds, the α -substitution being in both cases the same, with the β -substitution in one case a hydroxyl group and in the other case an aldehyde group.

The most striking difference between this group and the previous groups is that instead of having only one main band as in groups I and II there are two well-marked bands, which occupy relatively the same positions to each other. The α - β -dihydroxyindole shows an absorption band in the region of 2200 and 2300 A.U. characteristic of the absorption band in aliphatic compounds having hydroxyl groups. The hydroxyindole-aldehyde also shows this absorption band but, as is to be expected, it is not so pronounced. The aldehyde group substituted in the ring shows the same effect in intensifying the absorption as it does in the case of indole-aldehyde. The shift in the bands towards the red of hydroxyindole-aldehyde compared with dihydroxyindole is about 500 A.U., whereas the shift in the band of indole-aldehyde compared with indole is about 400 A.U. The agreement in the effect of the aldehyde is quite good. The aldehyde absorption band does not show up so distinctly in hydroxyindole-aldehyde as in indole-aldehyde owing no doubt to the fact that the position is already occupied by one of the main bands of the parent substance.

In the case of group IV nothing much can be said owing to the lack of a number of compounds on which to make comparisons. In these two cases the double bond has disappeared from the pyrrole ring and the change in the absorption completely destroys any resemblance to the other three groups. These compounds are not indole compounds but indolin derivatives.

Sincere thanks are due to Professor Hopkins for his kindness in purchasing for the Biochemical Department the very specialised apparatus required for this research.

REFERENCES.

- Ehrlich, Felix (1922). *Ber. deutsch. chem. Ges.* **55**, 865.
 Ellinger (1906). *Ber. deutsch. chem. Ges.* **39**, 2515.
 Friedländer and St Kielbasinski (1912). *Ber. deutsch. chem. Ges.* **45**, 3389.
 Henri, V. (1912). *Compt. Rend. Acad. Sci.* **155**, 457.
 — (1913). *Compt. Rend. Acad. Sci.* **156**, 551.
 Hopkins and Cole (1901). *J. Physiol.* **27**, 418.
 — (1903). *J. Physiol.* **29**, 451.
 Magina and Kotake (1922). *Ber. deutsch. chem. Ges.* **55**, 3865.
 Marschalk (1912). *Ber. deutsch. chem. Ges.* **45**, 583.
 Nencki (1889). *Monatsh. Chem.* **10**, 506.