96. *The Infra-red Absorptions of Asparagine and Glutamine.*

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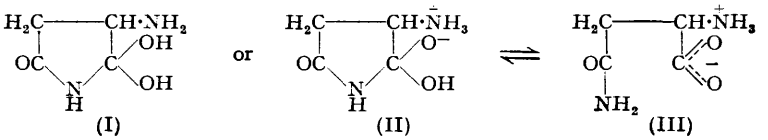
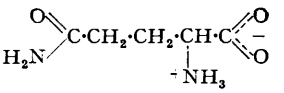
In contrast to a variety of other data recently summarised by Steward and Thompson the infra-red absorptions of asparagine and glutamine do not appear to differ significantly. In particular, the cyclic formula proposed by them for asparagine finds no support in the absorptions studied.

FROM a critical review, Steward and Thompson (*Nature*, 1952, **169**, 739) concluded that the differences in the behaviour of asparagine and glutamine are greater than would correspond to a pair of simple homologues; they proposed a new structure (*a*) for asparagine, and the properties of the two compounds were plausibly explained on the basis of this new formulation and the conventional straight-chain structure for glutamine (*b*). Steward and Thomson believe that asparagine in solution should be represented as a tautomeric system in which the equilibrium is far towards the ring form (II). Whether the new structure (I),

(*a*) 

(I) (II) (III)

(*b*) 

(IV)

having two (*gem*) hydroxyl groups in place of the conventional carboxyl structure, would have an acid strength at all comparable to the latter may be doubted but, even so, the zwitterionic form (II) must be considered as a possibility.* Part of the evidence in favour of the new asparagine structure consisted of unpublished infra-red observations by Dr. R. G. Gore. As we have been studying some of the characteristic groups in these structures (Orville Thomas, *Discuss. Faraday Soc.*, 1950, **9**, 339; Davies and Hallam, *Trans. Faraday Soc.*, 1951, **47**, 1170; Davies and Evans, *J. Chem. Phys.*, 1952, **20**, 342), crucial features in the absorptions of these compounds have been compared.

EXPERIMENTAL

The Grubb–Parsons S3. single-beam spectrometer equipped with silica and rock-salt prisms was used. Asparagine (a commercial sample of the hydrate from Messrs. Light) was dehydrated at 120°. We are indebted to Dr. Kenneth Bailey of the Biochemistry Department, Cambridge University, for providing us with a sample of glutamine: this might have contained a small amount of alanine but the presence of the latter would not, in any case, be expected to vitiate the comparisons being made.

Although thin films of glutamine could be prepared from the molten solid, this was not possible with asparagine without decomposition. Accordingly, mulls of the finely divided solids in carbon tetrachloride have been used. It was necessary to use different slit widths when the background and mull traces were being run in some instances: as a result, some of the detail found in the 6-μ region was unreal, arising as it did from the residual atmospheric water absorptions. These minor features are neglected in the following account. Saturated solutions of the compounds in D_2O (about 0·3M) were examined as capillary films between silver chloride windows. A small drop of concentrated hydrochloric acid (in H_2O) was added to produce an acid medium.

3-μ *Region.*—It can confidently be expected that structures (I) or (II), which contain hydroxyl groups, would absorb at higher frequencies in this region than would (IV), whose first fundamental band will be the N–H stretching frequency. In fact, both compounds, when anhydrous, show very little absorption until the latter band appears, giving rise to sharp peaks

* In a personal communication, Professor I. M. Klotz of Evanston, Ill., has pointed out the small difference in pK_a values, respectively 2·02 and 2·19 for asparagine and glutamine (see Cohn and Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, 1943, p. 84).

near 3354 cm.$^{-1}$ in asparagine and 3403 cm.$^{-1}$ in glutamine (Fig. 1) and further absorption at lower wave-numbers which merges in an ill-defined way into the CH region. In view of the absorptions of hydroxylic structures similar to (I) at wave-numbers higher than 3450 cm.$^{-1}$ (cf. Davies, *Trans. Faraday Soc.*, 1940, **36**, 1114), it is unlikely that the asparagine contains an OH group, the comparison with glutamine in Fig. 1 being virtually conclusive in this respect, The molecule of water present in asparagine hydrate shows up strongly with the absorption already having a shoulder at 3650 cm.$^{-1}$ whilst the N–H peak shifts only very slightly, from 3354 to 3358 cm.$^{-1}$ on hydration.

6-μ *Region.*—The range 1500—1750 cm.$^{-1}$ will be most significant as it includes the carbonyl and one of the carboxylate-ion stretching frequencies, ν (asymmetric) of $C\begin{matrix}O\\ \diagdown\\ \diagup\\ O\end{matrix}^{-}$. Glutamine shows three well-defined absorptions—at 1690, 1639, and 1586 cm.$^{-1}$—which very probably represent respectively (see Thomas, Davies, Hallam, and Evans, *locc. cit.*), ν (C=O) of the amide

FIG. 1. FIG. 2.

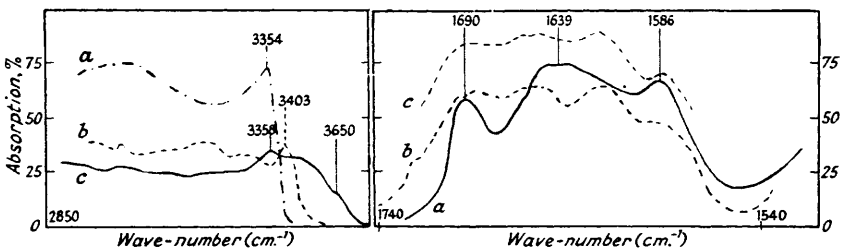


FIG. 1. *Film of* (a) *anhydrous asparagine,* (b) *anhydrous glutamine,* (c) *hydrated asparagine, all as mulls in* CCl_4.

FIG. 2. *Capillary film of* (a) *glutamine and* (b) *asparagine, and* (c) *thicker film of asparagine, all as mulls in* CCl_4.

FIG. 3. FIG. 4.

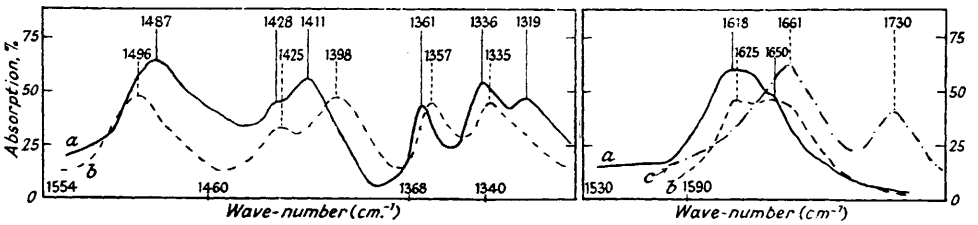


FIG. 3. *Capillary film of* (a) *glutamine and* (b) *asparagine, as mulls in* CCl_4.

FIG. 4. *Capillary film of saturated solution of* (a) *glutamine in* D_2O, (b) *asparagine in* D_2O, *and* (c) *asparagine in* D_2O *plus* HCl.

group, ν (*as*) of the carboxylate ion, and δ (NH_2) of the amide group (Fig. 2). The asparagine spectrum is not so well defined—possibly owing merely to the larger scatter of its powder in carbon tetrachloride—but three absorptions, approximately coincident with the above, have been recorded for different specimens. There is some suggestion—not always so marked as in Fig. 2—of a doublet structure to the carboxylate absorption at 1640 cm.$^{-1}$: this may arise from conditions in the crystalline solid. The δ (NH_2) frequency is now perhaps somewhat lower, at 1580 cm.$^{-1}$. It is by no means certain from our records that asparagine has an absorption centred near 1525 cm.$^{-1}$ as quoted from Gore's observations: in those instances where it appeared we are inclined to ascribe it to an atmospheric water absorption, and so it is not surprising that it also appears in some of the glutamine records.

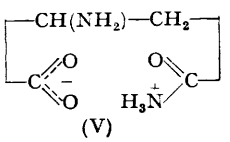
Apart from the general similarity shown in Fig. 2, the definite presence of the ν (*as*) carboxylate frequency at 1640 cm.$^{-1}$ in asparagine (cf. also the results in D_2O) again appears unfavourable to the correctness of structure (I) or (II), unless it is supposed that there is an accidental coincidence of one of the frequencies from those structures with this value: this, however, is rather unlikely, as a frequency as high as 1640 cm.$^{-1}$ almost certainly involves some double-bond character, and ν (C=O) of the amide group is already assigned to 1690 cm.$^{-1}$. Again,

the characteristic δ (NH_2) frequency appears to be present in asparagine. In the simplest terms, the asparagine absorption from 1500 to 1700 cm.$^{-1}$ is far too broad to arise solely from ν (C=O) and δ (N–H), and it is not clear what other frequency in (I) or (II) could contribute to this absorption.

6·6—7·8-μ *Region.*—For the mulls in carbon tetrachloride several well-defined absorptions are recorded in this range (Fig. 3). The differences between glutamine and asparagine are here no more than might be expected between the spectra of two homologues with the same type of structure. From the present observations the assignment of these frequencies cannot be made with certainty but, for immediate purposes, this is less significant than the apparent identity of the absorptions. Two modes δ (*as*) and δ (*s*) can be expected from the CH_2 groups : these may be 1490, and 1360 or 1335 cm.$^{-1}$, respectively. Another two absorptions are expected near 1400 cm.$^{-1}$ due to the ν (C–N) and ν (*s*) $C\overset{O}{\underset{O}{<}}^{-}$ modes : by comparison with other molecules (cf. Thomas and Hallam, *loc. cit.*) it would be plausible to assign the 1425 and 1428 cm.$^{-1}$ frequencies of asparagine and glutamine to the carboxylate group and the 1398 cm.$^{-1}$, 1411 cm.$^{-1}$ bands to ν (C–N). The presence of the former in asparagine does not conform to the new structure (I) or (II), or to Steward and Thompson's statement (*loc. cit.*, p. 742) that a band at 1420 cm.$^{-1}$ is absent in asparagine.

D_2O *Solutions.*—Because of the blurred absorption often found with solids, advantages are to be expected for the comparison of related compounds in solution. However, owing to their highly polar character, the present compounds make D_2O perhaps the only useful solvent for infra-red observations. In the carbonyl region, the absorptions are again similar, a broad band with the one centre at 1620 ± 5 cm.$^{-1}$ being found for both solutes (Fig. 4). For asparagine a second centre near 1650 cm.$^{-1}$ is probable, and a shoulder there is also seen in the glutamine absorption. These frequencies are ν (*as*) $C\overset{O}{\underset{O}{<}}^{-}$ and ν (C=O) of the amide group, respectively. This interpretation is fully confirmed by the effect of acidification, which converts $-COO^-$ into $-CO_2H$. Asparagine then very clearly shows ν (C=O) of the amide group at 1661 cm.$^{-1}$, and of the carboxylic group at 1730 cm.$^{-1}$. There appeared to be significant loss of glutamine on acidification (it is much more readily hydrolysed than asparagine, see Steward and Thompson, *loc. cit.*), but the addition of hydrochloric acid to its solution similarly led to the complete disappearance of the 1615 cm.$^{-1}$ band and the less certain (because weaker) appearance of bands at 1645 and 1729 cm.$^{-1}$. The D_2O solutions of the two solutes also failed to show any significant differences between 1530 and 1350 cm.$^{-1}$, *i.e.*, conforming, in their identity, with the solid absorptions.

The conclusions of this infra-red study are as follows : In all the features examined there is little significant difference between the asparagine and glutamine absorptions and, in particular, the presence of a normal carboxylate ion appears to be well established in the asparagine structure. Thus the particular solution of the differences between these compounds favoured by Steward and Thompson finds no support in the infra-red spectra. This may mean that some such suggestion as Dr. M. L. Huggins's, which involves a less radical structural difference between the homologues and ascribes a configuration, *e.g.*, (V), resulting from intramolecular interaction to asparagine, is more nearly the correct representation.



(V)

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