alcohol and 5 g. of digitonin dissolved in 200 cc. of hot ethanol was added. After standing overnight, the digitonide was filtered, dried, and decomposed by warming with pyridine in the usual manner. The product thus obtained was distilled in a high vacuum, collecting the portion distilling below 200°. This fraction was crystallized from methanol to give *allo*-pregnanediol-3(β),20(α); m. p. and mixed m. p., 214°.

The digitonin mother liquors were evaporated to about 30 cc. and 500 cc. of ether added. The digitonide was filtered, dried and then decomposed with pyridine. The crude product was distilled and crystallized from methanol. It proved to be β-equistanol, m. p. 133°. This was formed by the epimerization of α-equistanol, as the β-form had been removed previously.

The filtrate from the equistanol digitonide was evaporated to dryness and sublimed *in vacuo*. Two fractions were taken, one at 115–150° and the other at 150–200°. The lower boiling fraction after separation of some crystalline pregnanediol-3(α),20(α) was oxidized in acetic acid with an equal weight of chromic acid for thirty minutes. The oxidation mixture yielded mostly acidic material, and only a very small neutral fraction. The acidic fraction was extracted with ether and removed from the ethereal solution with sodium carbonate solution. Acidification of the alkaline solution yielded a crystalline acid which was recrystallized from ethanol to a constant m. p., 55°. Calcd. for $C_{18}H_{36}O_2$: neut. equiv., 284; found, 277.

Anal. Calcd. for $C_{18}H_{36}O_2$: C, 75.9; H, 12.7. Found: C, 76.2; H, 12.9.

Although polyhydroxy sterols probably belonging to the cortical series were present, the quantities were too minute for separation from the small amount of urine processed.

Hydrocarbon Fraction.—The ether soluble material from the preparation of the succinates of the sterols was distilled *in vacuo*. The portion distilling at 80–110° was dissolved in acetone and methanol was added. It was allowed to stand for two days at room temperature in an unstoppered flask. The crystalline material was recrystallized from acetone to give a product of m. p. 63°. Molecular weight calcd. for $C_{28}H_{58}$, 394; found (Rast), 393. It gave no depression in melting point with a hydrocarbon isolated from human pregnancy urine.

Anal. Calcd. for $C_{28}H_{58}$: C, 85.3; H, 14.8. Found: C, 85.2; H, 14.6.

Summary

An investigation of the sterols present in cows' pregnancy urine gave pregnanediol-3(α),20(α), *allo*-pregnanediol-3(α),20(α), and *allo*-pregnanediol-3(β),20(α), in approximately one-half the quantities present in human pregnancy urine. β-Equistanol was also isolated, and the presence of α-equistanol was demonstrated. The carbinol fraction upon oxidation gave an acid of the aliphatic series with a molecular formula of $C_{18}H_{36}O_2$. There was also isolated a hydrocarbon of the aliphatic series melting at 63° and having the molecular formula $C_{28}H_{58}$.

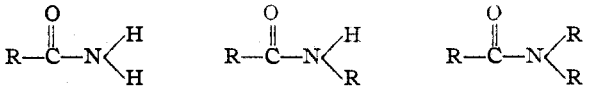
STATE COLLEGE, PENNA. RECEIVED AUGUST 8, 1938

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

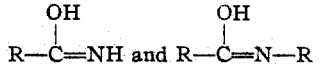
Infrared Absorption Studies. VI. Association in the Acid Amides and Oximes[1]

BY A. M. BUSWELL, W. H. RODEBUSH AND M. F. ROY

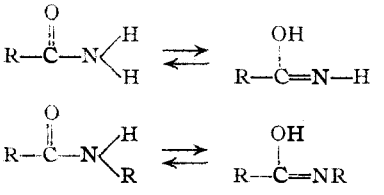
Structure of Acid Amides.—The classical structures assigned to amides and N-substituted amides are



However, certain reactions of the amides, *e. g.*, alkylation, indicate that compounds of the first two types above may have the alternative tautomeric structures



in that two isomeric reaction products are obtained in each case. Reactions of this type led to the belief that the amides probably existed in tautomeric equilibria of the two forms.



Attempts have been made to settle the question as to which of the two forms prevails by a study of the physical properties. Various authors[2] have found that the ultraviolet absorption spectrum indicates either tautomerism or the enolic form. Most of this work was in alcoholic solution but

(1) These studies are carried out with the aid of a grant from the Rockefeller Foundation. The main problem is the mechanism of hydration of biological substances ("bound water"). The present paper (VI) presents data on the absorption spectra of relatively simple organic nitrogen compounds whose functional groups resemble those found in proteins. Previous publications from this Laboratory dealing with association are II, *J. Chem. Phys.*, **5**, 501 (1937), and V, THIS JOURNAL, **60**, 2239 (1938).

(2) Ramart-Lucas and Gaunfeld, *Bull. soc. chim.*, [5] **4**, 478 (1937).

TABLE I

ACID AMIDES

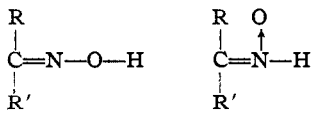
Amide	Molal concn.[a]	Cell length, cm.	Absorption maxima[b] CH μ	CH K	CH μ	CH K	NH μ	NH K	NH μ	NH K	Others μ	Others K	Others μ	Others K
Acetamide	0.001	10.18	3.30	7.5			2.925	27	2.83	27	3.145	10		
CH_3CONH_2	.002 (s)	5.07	3.30	7.5			2.925	27	2.83	29	3.145	17		
n-Valeramide	.002	10.18	3.45	41	3.37	68	2.92	23	2.83	26	3.14	19		
$C_4H_9CONH_2$	.004	5.07	3.45	41	3.37	70	2.92	23	2.83	24	3.14	27		
	.008 (s)	2.53	3.45	43	3.37	67	2.92	22	2.83	25	3.14	40		
	.002	10.18	3.47	46	3.40	73	2.90	19			2.97	7		
n-Cyclohexylacetamide	.008	5.07	3.47	46	3.40	73	2.90	17			2.98	8		
$CH_3CONHC_6H_{11}$	.032	0.640	3.47	46	3.40	73	2.90	16			2.99	23		
	.128	.1582	3.47	47	3.40	72	2.90	13			3.02	64	3.23	18
	.512	.0374	3.47	47	3.40	76	2.90	5			3.04	79	3.23	34
Acetanilide	.001	10.18	3.38	8	3.26	24	2.90	24						
$CH_3CONHC_6H_5$	.008 (s)	1.262	3.38	10	3.26	29	2.90	26			2.97	15		
Benzanilide	.0035 (s)	2.53	3.25	31			2.86	26						
$C_6H_5CONHC_6H_5$														
N,N-Diphenylacetamide	.008	1.262	3.38	4	3.25	29								
$CH_3CON(C_6H_5)_2$														
N,N-Diphenylbenzamide	.008	1.262	3.25	40										
$C_6H_5CON(C_6H_5)_2$														
N,N-Dimethylacetamide	.008	2.53	3.39	52										
$CH_3CON(CH_3)_2$														

[a] (s) indicates a nearly saturated solution.

[b] μ is the wave length of the absorption. K is the molal absorption coefficient.

Herzberg and Kölsch[3] have reported that the vapor of formamide shows no absorption characteristic of N—H or C=O. Data upon dipole moments and the Raman spectrum do not appear to offer any conclusive evidence upon this point. It is always possible, of course, to account for the reaction products assuming either formula to be correct. The only chance for a final decision upon the question would appear to be the determination of the characteristic infrared absorption due to hydrogen in the region from 2.6 to 3.6 μ. No report of any work in this region has appeared in the literature. Some results have been obtained in the region of the first overtone, which are however necessarily difficult to interpret.

The situation in regard to the oximes is somewhat similar to that of the amides. There are two structures which are compatible with the chemical evidence.



There is of course a possibility of tautomerism and the final decision as to structure must be made from a study of physical properties. Here again Freymann and Freymann[4] have studied the infrared absorption in the region of the harmonics with results which indicated the presence of the hydroxyl group.

Experimental

The infrared spectrograph used for this work has been described in previous publications in this series. The compounds used in this work were prepared and carefully purified in this Laboratory by Mr. J. R. Downing. The data in every case represent the ratio of the absorption of a solution in carbon tetrachloride to the absorption of the pure solvent. The molal absorption is calculated as given by the formula

$$\frac{1}{cd} \log \frac{I_0}{I}$$

and the product $c \times d$ is maintained constant for each compound in order to avoid deviations from Lambert's law. The chief difficulty was the low solubility of amides and oximes in carbon tetrachloride. The higher homologs were found to be somewhat more soluble. For those amides for which no curve is shown the data for the principal absorption peaks are included in a table. Curves are given for all of the oximes which were studied.

The Amides.—The high melting and boiling points of the amides have long been recognized as evidence for a high degree of association. Kumler[5] has pointed out that this evidence as well as that of cryoscopic data point to a marked differ-

(3) G. Herzberg and R. Kölsch, *Z. Elecktrochem.*, **39**, 672 (1933).

(4) M. and R. Freymann, *Bull. soc. chim.*, [5] **4**, 944 (1937).

(5) W. D. Kumler, THIS JOURNAL, **57**, 600 (1935).

ence in the behavior of the amides when the hydrogens attached to the nitrogen are replaced by alkyl groups. This evidence points to hydrogen bonding as the mechanism by which association takes place. Recently Zellhoefer, Copley and Marvel[6] have found that the solubility of chloroform varies in the different amides in such a way as to agree closely with the degree of association to be expected.

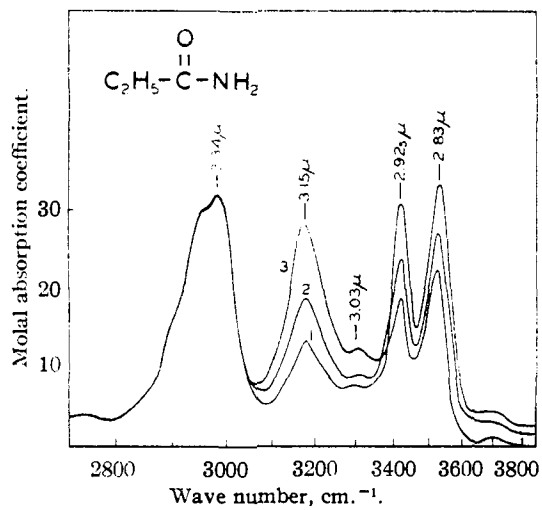


Fig. 1.—Propionamide: (1) 0.002 *M* in 10.18-cm. cell. (2) 0.004 *M* in 5.07-cm. cell. (3) 0.008 *M* in 2.53-cm. cell.

The first curve shown, Fig. 1, is that for propionamide which because of somewhat greater solubility permits a greater range of concentration than acetamide. The C–H absorption at 3.34 μ remains constant over the concentration range while a marked variation with concentration is shown in the region of N–H absorption and there is no indication of any O–H absorption. One may say, therefore, that in the ordinary amides in carbon tetrachloride solution there is no tendency toward enolization. The NH_2 group may be expected to show modes of vibration similar to that of the water molecule.[7] There are two modes involving the stretching of the N–H bonds which are infrared active. One of these is symmetric with respect to the hydrogens and one anti-symmetric. In water these frequencies are at 2.67 and 2.76 μ, respectively. Here we find the N–H frequencies at 2.83 and 2.92 μ. As the concentration is increased the association through hydrogen bonding increases and the absorption at 3.15 μ characteristic of hydrogen bonded N–H increases. The unbonded N–H peaks are reduced in intensity but otherwise unmodified. The solubility is limited so that the association is not complete at the highest concentration obtainable but one cannot doubt that at higher concentrations in another solvent for example, the absorption characteristic of the unbonded N–H would disappear completely.

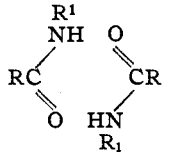
This behavior is perhaps best accounted for by assuming that when the molecule enters a polymer both hydrogens are involved in the bond formation. Benzamide does not show any particular difference except that the relative intensity of the two peaks in the N–H absorption is altered. The intensities depend indirectly upon the dipole moments associated with the N–H bonds and also the angle between them, and these are evidently slightly altered by the introduction of the phenyl group. The C–H absorption at 3.25 μ which is characteristic of aromatic molecules appears to vary somewhat, but this may be due to the overlapping N–H bond peak. The absorption curves for N-ethyl acetamide in Fig. 3 show interesting variations from those for the unsubstituted amides. The C–H peak is the same as before and only a single peak is to be expected for the N–H absorption and this appears as might be expected intermediate in position between the two peaks of Fig. 1. The peak at 3.22 μ may be assumed to correspond to the peaks at 3.15 μ in the other cases and hence to represent the hydrogen bonded N–H but the peak at 3.03 μ corresponds exactly to that of a hydrogen bonded hydroxyl. Furthermore, the great increase in intensity is strongly suggestive of a hydroxyl which absorbs more strongly than does an N–H group. We believe therefore that this absorption indicates the presence of the enolic form $CH_3C(OH)=NC_2H_5$. This form only exists in the polymer at higher concentrations since there is no absorption in the region 2.8 μ which would be characteristic of unbonded hydroxyl. It may be said therefore that at higher concentrations N-ethylacetamide is largely associated and that a considerable fraction (one-half or more) of the molecules so associated exist in the enolic form. It should be noted of course that N,N-dimethylacetamide shows no absorption except that due to C–H and no physical behavior indicating association.

(6) G. F. Zellhoefer, M. J. Copley and C. S. Marvel, THIS JOURNAL, **60**, 1337 (1938); also an article to appear soon.

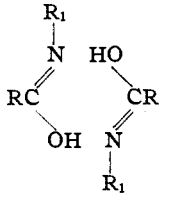
(7) E. F. Barker and W. W. Sleator, *J. Chem. Phys.*, **3**, 660 (1935).

To summarize we may say that while the ordinary amides show a strong tendency toward association (much greater than the alcohols at low concentration) the monosubstituted amides show a smaller tendency, and the disubstituted amides do not associate at all. This conclusion is confirmed by the various physical properties, cryoscopic behavior, behavior as a solvent, etc. In the case of the unsubstituted amides the dielectric constant and other properties suggest the formation of polymers of indefinite molecular weight, similar to those which occur in the alcohols. In the monosubstituted amides the tendency toward association is less strong and one may assume that a considerable fraction of the molecules are associated to form dimers. Dr. M. J. Copley of this Laboratory informs us that the solubility of chloroform in N-ethylacetamide could be accounted for by assuming the pure liquid to consist largely of dimers.

While there are certain well-recognized conditions necessary for enolization to take place in a molecule, we believe that one which has not been sufficiently emphasized is that *the shift of the mobile hydrogen usually involves the formation of a hydrogen bond.* An isolated amide molecule in carbon tetrachloride has no opportunity for forming a hydrogen bond. (The case would of course be different if the solvent were alcohol.) Dr. M. J. Copley has suggested to us that if a dimer is formed with bonds N–H–O similar to the carboxylic acids



then by a shift of hydrogens the structure



is easily obtained and the second structure may easily be the more stable. It must be remembered that our evidence shows that enolization takes place only in the associated molecules and it is only in the dimers with N–H → O bonds that a simple mechanism has been suggested. In a chain polymer, for example, enolization would involve a molecular rearrangement equivalent to ionization of the hydrogen, which is hardly to be expected to take place in a carbon tetrachloride solution. In the absence of other suggestions as to the mechanism of enolization one is forced to assume the existence of dimers in the monosubstituted amides and, as has been said above, this assumption is favored by the other known properties of these substances in the liquid state. Any attempt to explain why the unsubstituted amides do not form dimers or if they do form these, why these dimers do not enolize, would of course be highly speculative. One of course may reason by analogy with formic acid where in the pure liquid the dimers give way to polymers, presumably because of the bonding action of the hydrogen attached to carbon. In an unsubstituted amide the R_1 groups shown above would be replaced by hydrogens and it is easy to suggest ways in which these additional hydrogens might interfere with the formation of stable dimers. It should be noted of course that there is a slight absorption at 3.03 μ in Figs. 1 and 2 suggestive of a slight tendency toward enolization. One may predict that the reactions of the acid amides will under comparable conditions confirm the hypothesis of enolization that has been proposed above.

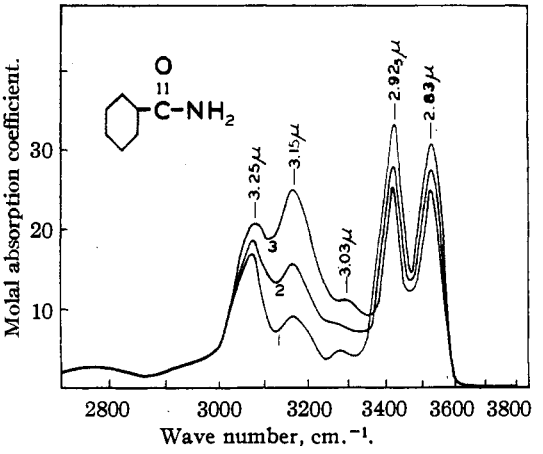
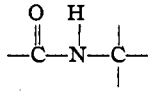


Fig. 2.—Benzamide: (1) 0.0005 *M* in 10.18-cm. cell. (2) 0.001 *M* in 10.18-cm. cell. (3) 0.002 *M* in 5.07-cm. cell.

It should be noted that the essential group in the N-substituted amides is the same as that of the peptide linkage in the proteins, *viz.*



The absorption curve of gelatin[8] shows such

(8) A. M. Buswell, Karl Krebs and W. H. Rodebush, THIS

striking similarities that one can scarcely doubt that precisely the same type of hydrogen bonding is present in both cases. One would assume, of course, that the hydrogen bonding occurs as a cross linkage between two polymeric chains or perhaps between parts of the same chain.

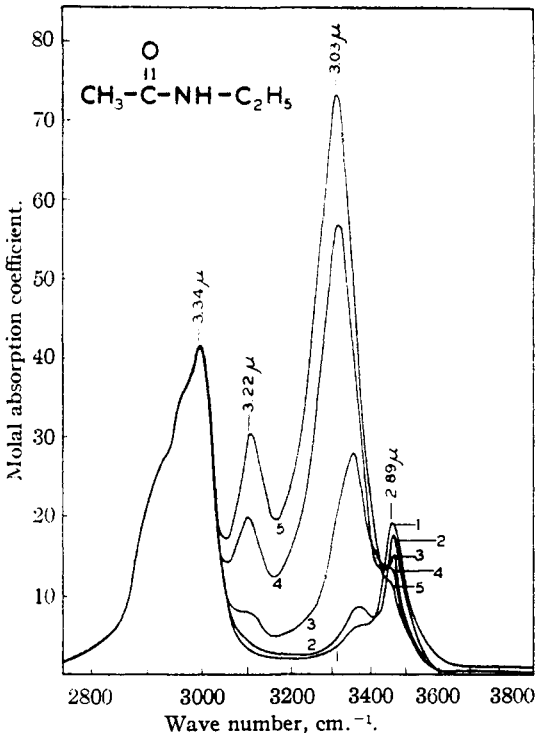
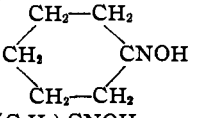


Fig. 3.—N-Ethylacetamide: (1) 0.002 *M* in 10.18-cm. cell. (2) 0.008 *M* in 5.07-cm. cell. (3) 0.032 *M* in 0.640-cm. cell. (4) 0.128 *M* in 0.1582-cm. cell. (5) 0.512 *M* in 0.0374-cm. cell.

The Oximes.—In order to avoid complications which might arise from possible geometrical isomerism, only symmetrical ketoximes were studied. In Figs. 4, 5, 6 and 7 the absorption curves are given at various concentrations for the following compounds.

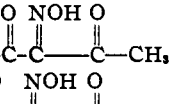
Acetoxime $(CH_3)_2CNOH$

Cyclohexanone oxime

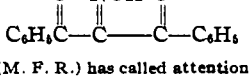


Benzophenone oxime $(C_6H_5)_2CNOH$

The oxime of dimethyl triketone



The oxime of diphenyl triketone



JOURNAL, **59**, 2603 (1937). One of us (M. F. R.) has called attention to the fact that the enolized dimer should dissociate into enolized monomers. This does not happen and the hypothesis of enolization cannot be considered as fully substantiated until the absence of the enolic form in the monomers is accounted for.

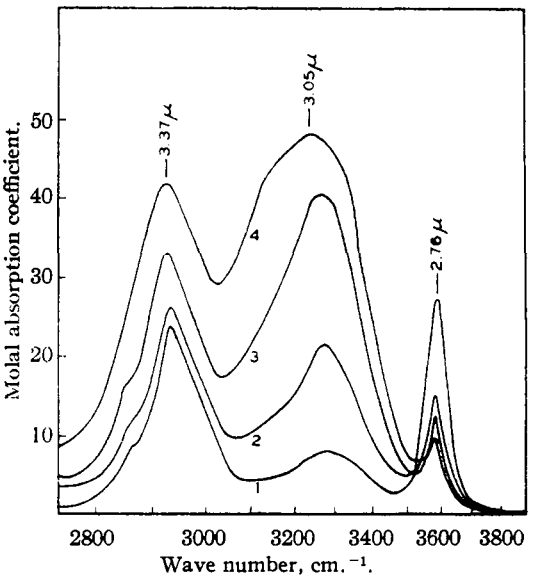


Fig. 4.—Acetoxime: (1) 0.001 *M* in 10.18-cm. cell. (2) 0.004 *M* in 10.18-cm. cell. (3) 0.016 *M* in 2.53-cm. cell. (4) 0.064 *M* in 0.640-cm. cell.

The absorption peak at 2.76 μ in Figs. 4, 5 and 6 is clearly indicative of the hydroxyl group which is to be expected in the usually accepted formula for oximes. There is no indication whatever of an N–H absorption. As the concentration is in-

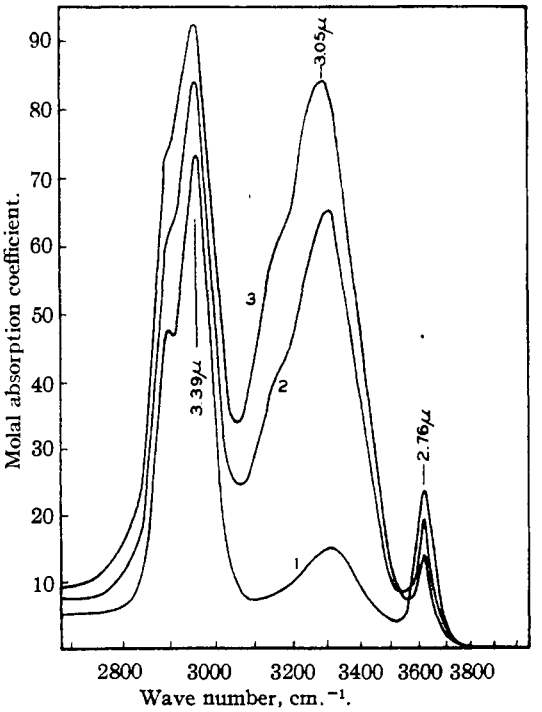


Fig. 5.—Cyclohexanone oxime: (1) 0.0178 *M* in 10.18-cm. cell. (2) 0.0285 *M* in 0.640-cm. cell. (3) 0.1139 *M* in 0.1582-cm. cell.

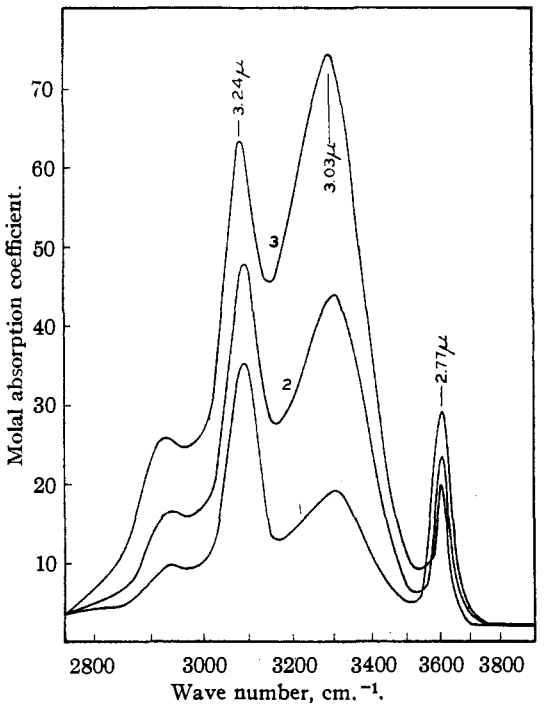


Fig. 6.—Benzophenone oxime: (1) 0.002 *M* in 10.18-cm. cell. (2) 0.008 *M* in 2.53-cm. cell. (3) 0.032 *M* in 0.640-cm. cell.

creased the "association peak" characteristic of hydrogen bonding appears at about 3.0 just as in the alcohols.[9] All this is quite to be expected. The startling thing about these curves is that the molal absorption due to C–H increases with increasing concentration in spite of the fact that the product of concentration times cell length was maintained constant. This behavior has been noted already in the case of benzamide which shows C–H absorption and we are by no means certain how to account for it. There is of course the possibility that the oximes form unsymmetrical dimers so that all C–H vibrations remain active. (In a symmetrical dimer as acetic acid only the anti-symmetric C–H vibrations are active.) Under these conditions because of the augmented dipole moment, the change in moment associated with transition might be increased sufficiently to account for the increased absorption. A simpler explanation of course of the increasing C–H absorption is that the absorption due to the hydrogen bonding overlaps the C–H region. In view of the acidic character of the oximes, this is a reasonable assumption. In the carboxylic acids the absorption extends over a very broad interval. The unbonded hydroxyl frequency of the oximes is at 2.77 μ which is very near the unbonded hydroxyl wave length of acetic acid.

(9) A. M. Buswell, V. Deitz and W. H. Rodebush, *J. Chem. Phys.*, **5**, 501 (1937).

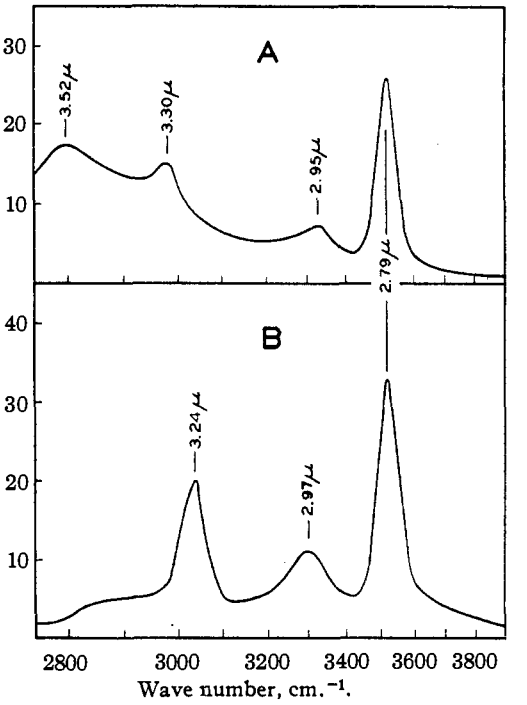


Fig. 7.—A. Oxime of dimethyl triketone: 0.002 *M* in 10.18-cm. cell. B. Oxime of diphenyl triketone: 0.002 *M* in 10.18-cm. cell.

In all of the results so far we have no direct evidence as to whether the bonding is to oxygen or nitrogen (N–H → O or N–H → N) since either is generally possible. In an attempt to distinguish between these possibilities the absorption for the two triketone oximes was measured, Fig. 7. Here while the solubility is too low to permit of much association, there is reason to believe that the absorption at 2.95–2.97 μ is indicative of intermolecular hydrogen bonding which is presumably OH → O. The absorption at 2.79 is of course the unbonded hydroxyl and there is no evidence of chelation.

Summary

Infrared absorption has been studied for a number of acid amides and oximes. Association due to hydrogen bonding is observed and in the case of monosubstituted amides association is accompanied by a considerable amount of enolization. Attention is called to the striking similarity of the absorption due to association in the substituted amides and that due to the peptide group in gelatin.

URBANA, ILLINOIS RECEIVED JULY 11, 1938