133. *Infrared Spectra and Dehydrogenase Activity of Isatin Derivatives.*

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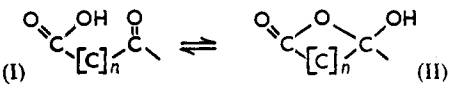
The vibrational spectra of 7-methylisatin-4-carboxylic acid and related compounds have been examined. The lactol form of the acid is present in the solid state; normal carboxylic acid dimerisation occurs in concentrated solution, and an intramolecularly bonded form exists in dilute solution. Various hydrogen-bonded forms occur in the derivatives, and the possible relation between these structures and the dehydrogenase activity is discussed.

EARLIER publications [1,2] have related dehydrogenase activities of substituted isatins to the stretching frequencies of the carbonyl groups. This work has now been extended by an analysis of the spectra of the highly active 7-methylisatin-4-carboxylic acid and its derivatives.[3] The variety of structures which may be written for the acid include the keto–lactol tautomers and two hydrogen-bonded forms involving the carboxyl group. Numerous examples of keto–lactol tautomerism have been described in the literature, many of them occurring in γ-ketonic acids (I; $n = 2$). Various of these compounds have been shown to be biologically active, such as *o*-benzoylbenzoic acid,[4] and the weakly antibacterial mould products, penicillic [5,6] and gladiolic acid.[7] Such compounds may usually be recognised by the formation of two series of esters, the normal carboxylic esters and the



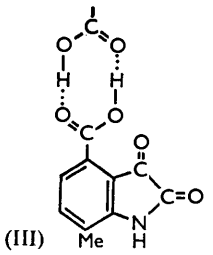
(I) (II)

hydroxy-lactonic esters derived from (I) and (II) respectively, although benzil-*o*-carboxylic acid forms only the normal methyl ester. Ultraviolet absorption spectra have been extensively used in the elucidation of the structures of γ-ketonic acids,[8] and more recently infrared spectroscopy has been used.[9] For isatincarboxylic acids, the interpretation of infrared data is difficult because the carbonyl stretching frequency of the carboxyl group overlies, and in some cases merges with, the α- and the β-carbonyl frequency, but the use of several reference compounds has made band assignment reasonably certain. The compounds under investigation are insoluble in non-polar solvents such as carbon disulphide and carbon tetrachloride, and in weakly polar solvents such as chloroform, but are soluble in dimethylformamide and dioxan which, unfortunately, are not satisfactory. However, a suitable compromise was found in the use of *sym*-tetrachloroethane.

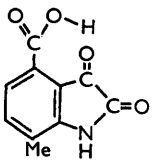
[1] O'Sullivan and Sadler, *J.*, 1956, 2202.
[2] *Idem, Arch. Biochem. Biophys.*, 1957, **65**, 243.
[3] Mix and Krause, *Chem. Ber.*, 1956, **89**, 2630.
[4] Sexton and Templeman, *Nature*, 1948, **161**, 974.
[5] Birkinshaw, Oxford, and Raistrick, *Biochem. J.*, 1936, **30**, 394.
[6] Raphael, *Nature*, 1947, **160**, 261.
[7] Brian, Curtis, Grove, Hemming, and McGowan, *ibid.*, 1946, **157**, 697.
[8] Shaw, *J. Amer. Chem. Soc.*, 1946, **68**, 2510.
[9] Grove and Willis, *J.*, 1951, 877.

Typically, the infrared spectra of substituted isatins in chloroform and tetrachloroethane possess a broad band between 3500 and 3200 cm.$^{-1}$, which contains two maxima, a fairly sharp peak (see Table 1) at about 3450 cm.$^{-1}$ and a broader maximum near 3230 cm.$^{-1}$; the former is an N–H stretching frequency and the latter results from the intermolecular NH • • • O=C bonding, which exists in all but a few ring-substituted isatins.[1] Two carbonyl maxima normally occur near 1740 and 1755 cm.$^{-1}$; the absorption of the lower frequency is attributed to the β-carbonyl group and is sharp and intense, whilst the α-carbonyl absorption is always slightly less intense and, although usually present as a well-defined maximum, is occasionally evident only as a shoulder.[1] In general, the separation of the two carbonyl peaks is less distinct in 4- and 6-substituted compounds, the resonance structures presumably tending to equalise the character of the two carbonyl groups. These compounds are the least soluble in solvents of low polarity and from the character of the 3230 cm.$^{-1}$ absorption appear to possess very strong intermolecular links.[1] A 1620 cm.$^{-1}$ band is present which is always comparable in intensity with the carbonyl frequencies and in a few cases is divided into two high peaks with maxima respectively slightly above and below 1620 cm.$^{-1}$. Some compounds possessing 4-substituents show a maximum at a slightly lower frequency, in accordance with the usual behaviour of vicinally substituted benzene derivatives.

7-Methylisatin-4-carboxylic acid (III) in concentrated solution in *sym*-tetrachloroethane shows only the broad bonded O–H absorption at 2800—3200 cm.$^{-1}$, characteristic of the carboxylic acid dimer superimposed on the C–H stretching absorption at 2850 cm.$^{-1}$; the NH peaks are not clearly defined. The α- and the β-carbonyl stretching frequency



(III)



(IV)

(V)

occur at 1760 and 1735 cm.$^{-1}$ respectively, both being very weak in comparison with the intense absorption at 1670 cm.$^{-1}$. This is slightly lower than the typical aromatic carboxylic acid dimer band at 1685 cm.$^{-1}$ and is in agreement with Flett's findings [10] that aromatic acids which are capable of internal hydrogen-bonding have the dimer band below 1680 cm.$^{-1}$. The band at 1590 cm.$^{-1}$ is an aromatic frequency. On dilution, the 1670 cm.$^{-1}$ peak steadily diminishes and a new intense peak appears at 1705 cm.$^{-1}$ due to the monomeric form (IV), which is intramolecularly bonded as no absorption at 3600 cm.$^{-1}$ characteristic of free OH groups is shown. The β-carbonyl peak no longer occurs as a separate entity, but weak absorption is still shown at 1760 cm.$^{-1}$. The amide corresponding to this acid shows typical free and bonded N–H stretching frequencies at 3500 and 3340 cm.$^{-1}$ respectively. The C–H stretching frequency occurs as a well-defined maximum at 2900 cm.$^{-1}$ and the α- and the β-carbonyl group give rise to a fairly broad band at 1735 cm.$^{-1}$. The amide I band occurs as a strong peak at 1710 cm.$^{-1}$. Associated and unassociated forms may give rise to two amide II bands,[11] which appear as two split maxima at 1641—1566. The aromatic frequency bands appear at 1560 and 1615 cm.$^{-1}$.[12] The situation is similar in the case of the propylamide, except that the N–H absorption closely approximates to that of isatin itself; presumably the amide N–H is bonded to the β-carbonyl group, the stretching frequency of which could be masked by the strong amide I band at 1720 cm.$^{-1}$;

[10] Flett, *J.*, 1951, 962.
[11] Richards and Thompson, *J.*, 1947, 1248.
[12] Bomstein, *Analyt. Chem.*, 1953, **25**, 512.

alternatively, the α- and the β-carbonyl stretching frequency may form a single band at 1762 cm.$^{-1}$. The amide II band is clearly defined as a single maximum at 1650 cm.$^{-1}$, the aromatic frequencies appearing at 1620 and 1580 cm.$^{-1}$. The dipropylamide, in accord with other *NN*-disubstituted amides, has no amide II band; again the amide I band at 1735 cm.$^{-1}$ may have the β-carbonyl stretching frequency superimposed, or both isatin carbonyl groups may give rise to the single band at 1755 cm.$^{-1}$.

5-Carboxymethylisatin was chosen as a reference compound as it is isomeric with 7-methylisatin-4-carboxylic acid but may form only intermolecular hydrogen-bonds. As this compound is a substituted phenylacetic acid its spectrum bears a closer relation to that of a saturated carboxylic acid than to that of an aromatic acid.[10] The strong absorption at 1708 cm.$^{-1}$ is typical of the dimeric form. The absence of free OH absorption confirms this, the bonded OH of the carboxylic acid dimer giving rise to the typical broad band with a weak maximum at 2850 cm.$^{-1}$ where it is superimposed on the C–H stretching frequency. At the concentration used, which was the limit of solubility of this compound, the carbonyl frequencies did not appear as separate entities, but a saturated solution of the more soluble methylated derivative, 5-carboxymethyl-1-methylisatin, showed strong carbonyl absorption at 1735 cm.$^{-1}$, of equal intensity to that of the 1708 cm.$^{-1}$ dimer band. 1-Methylisatin also possesses a single carbonyl peak.[1] The bonded OH absorption of 5-carboxymethyl-1-methylisatin was similar to that observed with the unmethylated 5-carboxymethylisatin. The last two compounds in Table 1, the ethyl esters, were included

TABLE 1. *Infrared spectra of substituted isatins in* sym-*tetrachloroethane.*

Substituent										
None [a]	—	1619	—	—	—	1736	1755sh	2920	3230	3420
4-CO_2H-7-Me [a]	1590	—	—	1670w	1705s	—	1760w	—	—	—
4-CO_2H-7-Me [b]	1590	—	—	1670s	—	1735w	1760sh	2850	(2800—3200)	
4-CO·NH_2-7-Me [a]	1560	1615	1641—1655	—	1710s	1735	—	2900	3340	3500
4-CO·NHPr-7-Me [a]	1580	1620	1650	—	1720	—	1762	2920	3300	3425
4-CO·NPr_2-7-Me [a]	1595	1625	—	—	—	1735	1755	2920	—	3425
5-CH_2·CO_2H [a]	—	1625w	—	—	1708s	—	—	—	—	—
5-CH_2·CO_2H-1-Me [a]	—	1625	—	—	1708	—	—	—	—	—
,, [b]	1600	1625	—	—	1708	1735br	—	—	—	—
5-CH_2·CO_2Et-1-Me [a]	—	1625w	—	—	1710	—	—	2920	—	—
5-CH_2·CO_2Et [a]	—	1625w	—	—	1710	—	—	2920	3230	3420

[a] 10^{-3}M-Solution in 0·35 mm. cell. [b] Saturated soln. in 0·35 mm. cell. w = weak, s = strong, br = broad, sh = shoulder.

to show that overtones were not responsible for the absorption in the bonded OH region; the bonded and free NH stretching frequencies of the last compound were similar to those of isatin; no significant absorption was detected in the 3 μ region for the penultimate compound.

The infrared spectral data obtained from the compounds in potassium bromide discs are presented in Table 2. The relation to the spectra obtained from solutions is quite

TABLE 2. *Infrared spectra of substituted isatins (potassium bromide discs).*

Substituents									
None	—	1615	—	—	1730	2900	3200	—	3420
4-CO_2H-7-Me	1595	1620	—	1700w	1740s	2900	3150	—	3400
4-CO·NH_2-7-Me	1560	1624	1650	1698	1728	2850	3190	3300	3400
4-CO·NHPr-7-Me	1580	1615	1630	1710	1755	2900	3190	—	3400
4-CO·NPr_2-7-Me	1590	1628	—	—	1745	2900	3200	—	3400
5-CH_2·CO_2H	—	1621	—	—	1730		2800—3200		
5-CH_2·CO_2H-1-Me	1605	1625	—	1721	1740		2800—3200		
5-CH_2·CO_2Et-1-Me	—	1625	—	1715	1735/1748	—	—	—	—
5-CH_2·CO_2Et	—	1623	—	1723	1738/1745	2900	3200	—	3420

clear, and band assignments are similar, but the small frequency fluctuations which are always associated with solid-state spectra are present. The variability of the spectra of substituted benzoic acids in alkali halide discs has been discussed by Farmer,[13] but with the particular preparative conditions used the 7-methylisatin-4-carboxylic acid disc should exhibit the dimer band. This band is, however, absent and a new intense peak appears at 1740 $cm.^{-1}$. As a similar band has been described for the lactol form of benzil-*o*-carboxylic acid,[9] it is possible that the lactol form (V) of 7-methylisatin-4-carboxylic acid is the preferred configuration in the solid state, rather than the dimer (III).

It is not possible, in the case of 7-methylisatin-4-carboxylic acid, to relate dehydrogenase activity to the carbonyl stretching frequencies as the latter do not occur as discrete entities. Nor may the high catalytic activity [3] be compared with the σ values for the substituents as the carboxyl group is *ortho* to the β-carbonyl group. However, attention has already been drawn to the possible relation between the unusual dehydrogenase activity of isatins substituted with acid groups in the 4-position and *intra*molecularly hydrogen-bonded structures which may occur in these compounds.[14] This is supported by our results, as under conditions approximating to those used in the measurements of catalytic activity, 7-methylisatin-4-carboxylic acid is shown to exist predominantly in an *intra*molecularly hydrogen-bonded form. But there is obviously no simple relation between bonding propensity and the dehydrogenase activity of 4-substituted isatins, as both the simple amide and the propylamide possess *intra*molecular hydrogen-bonds, yet there is considerable discrepancy between their relative dehydrogenase activities (see

TABLE 3. *Times required for the decolorisation of Methylene Blue in the oxidation of alanine.*

Catalyst: Isatin	Concn. (10^{-5}M)	Time (min.)	Catalyst: Isatin	Concn. (10^{-5}M)	Time (min.)
Unsubst.	2	59	4-CO·NPr_2-7-Me ...	2	353
4-CO_2H-7-Me	2	4·5	4-CO·NH_2-7-Me ...	2	Not decolorised
4-CO·NHPr-7-Me	2	50	4-CO·NH_2-7-Me ...	5×10^{-5}	1,002

Table 3). The very low activity of the unsubstituted amide is difficult to explain. No spectral evidence has been obtained to support the suggestion that in this case a stable intermediate is formed between the substituted isatin and the amino-acid involved in the dehydrogenation, although this would provide a simple explanation of the phenomenon. Generally, the interpretation of *ortho*-substituent effects is complex, and a more extensive investigation will be required if the anomalous results for these 4-substituted isatins are to be fully explained.

Experimental.—Spectra were determined by using a Perkin-Elmer 21 double-beam recording spectrometer fitted with a rock-salt prism.

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INSTITUT FÜR KATALYSEFORSCHUNG, ROSTOCK, DER DEUTSCHEN AKADEMIE DER WISSENSCHAFTEN ZU BERLIN. [*Received, October 7th,* 1958.]

[13] Farmer, *Spectrochim. Acta*, 1956, **8**, 374.
[14] Mix, Krause, and Reihsig, *J. prakt. Chem.*, 1958, **6**, 174.