REVIEW OF LITERATURE
A. Biological properties of 2,3-dioxo-indoline (isatin) and its derivatives

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A. Biological properties of 2,3-dioxoindolines (isatin) and its derivatives

A survey of biological and related properties of 2,3-dioxo-indolines is given below. The literature has been compiled up to the end of April, 1973.

(a) Enzyme-like activity

Oxidation of L-amino acids and the non-enzymatic dehydrogenase activity

Traube\textsuperscript{11} seems to have been the first to report in 1911 the oxidation of L-amino acids by isatin and by allied molecules like alloxan, ninhydrin and quinones to the corresponding one carbon atom lower aldehydes, CO\textsubscript{2} and NH\textsubscript{3}. At that time very little was known about the fine structure of proteins and enzymes. Experiments with similar chemical models, particularly in the hands of Bergman\textsuperscript{12} had led to interesting results in the chemistry of proteins.

On this basis Langenbeck (1927)\textsuperscript{13} felt that it was quite conceivable that similar model experiments might also furnish new clues to enzyme chemistry. This idea coupled with the above observation of Traube prompted Langenbeck and his coworkers during the years 1927-1956 to embark upon a comprehensive study of the dehydrogenase-like activity of isatin. The mechanism of oxidation (dehydrogenation) of L-amino acids suggested by Langenbeck\textsuperscript{13} was based on the reversible reduction of isatin to isatide in which atmospheric oxygen or methylene blue could serve as hydrogen acceptor.
Langenbeck (1928) reported that dehydrogenase activity of isatin was relatively small as compared to that of isatin 5-sulphonic acid. The catalytic activity of isatin was found to increase appreciably at temperatures between 70° to 100° in comparison to that at room temperature. Although N-methyl isatin was slightly less active, isatin 1-acetic acid was definitely a weaker catalyst. On the other hand, 5-halogeno (chloro, bromo or 5,7-dibromo) and 5-sulphonic acid derivatives were more active than the parent compound. Langenbeck (1937a) observed that this activity of isatin was also exhibited by 3-amino-naphthoxindole. Amongst these compounds, 6-hydroxy and 6-methyl derivatives were more active than the parent compound.

In a later communication Langenbeck and coworkers (1937b) observed that the catalytic effect, which they had mentioned as artificial carboxylase and artificial dehydrase reactions,
was very much enhanced in dilute pyridine solution containing a little acetic acid. A further increase in the catalytic activity was affected by introducing a carboxyl group in 4 and 6 positions. The carboxylic acid was found to be 3 times more active than the yellow enzyme of Warburg and Christian. On the basis of these observations they pointed out that these compounds could justifiably be called artificial enzymes. These authors (1956)\textsuperscript{17} later reported the dehydrogenase activity in relation to oxidation-reduction potentials and molecular weights of some complex isatins, such as phenyl-, styryl-, indolo- and coumarino-isatins.

Abderhalden (1948)\textsuperscript{18} observed that isatin, and also alloxan and ninhydrin, could oxidize L-amino acids to the corresponding lower aldehydes which were isolated as the dinedon derivatives. He also studied the action of isatin on polypeptides.

Giovannini and Portman (1948,57)\textsuperscript{19-21} attributed the dehydrogenase activity of isatin to its 3-keto group and further found that this activity could be modified by proper substitution in the benzene ring. They observed that the activity was enhanced by substitution in the 4 and 6 positions. Isatin 4- and 6-carboxylic acids in particular
were found to be far more effective as catalysts than the parent compound.

Mix and Krause (1956)\textsuperscript{22} tested the dehydrogenase activity of 4-substituted isatins and $N$-(7-methyl isatin-4-carbonyl) amino acid ethyl esters substituted in the 4-position against methylene blue in 70\% dimethyl-formamide at 40\degree. They found that introduction of a methyl group at position 7 had no effect on the catalytic activity of isatin. In a later communication Mix, Krause and Reihrig (1958)\textsuperscript{23} tested acid amides of 7-methyl isatin-4-carboxylic acid and other derivatives of the carboxylic group. 7-methyl-4-carboxylic amide of isatin was found to be the most active catalyst; the tertiary amides were less active. Hydrogen bonding was discussed and the greater activity of these compounds was attributed to hydrogen bonding.

O'Sullivan and Sadler (1957)\textsuperscript{24} implicated both the carbonyl groups of isatin in the dehydrogenase activity. They (1957)\textsuperscript{25} further examined the $\sigma$ values and the stretching frequencies in infra-red spectra in relation to catalytic activities and discussed their implications in predicting the pharmacological actions of such compounds.
Sadler, Mix and Krause (1969) studied the infra red spectra of isatin derivatives in relation to dehydrogenase activity. They tried to correlate the activity of 7-methyl isatin 4-carboxylic acid with its various possible structures and discussed the role of hydrogen bonding in this compound.
Cassebaum (1954) observed that the electrochemical behaviour of isatin, isatin 4-carboxylic acid, isatin 5-carboxylic acid, isatin 6-carboxylic acid, isatin 7-carboxylic acid, 5-phenyl isatin, 6-phenyl isatin, 5-styryl isatin, 6-styryl isatin, 5-phenyl isatin, 4-sulphonic acid, 4,5 benzo-isatin, 6,7-benzo isatin and 7-bromo-5,6-benzo isatin were related to their dehydrogenase activity. These studies were extended by Cassebaum in 1958.

Cassebaum and Liedel (1960) studied the dependence of catalytic activity on polar and steric effects of substituents in isatin. The role of hydrogen bonding and solvents were also examined.

(b) Enzyme inhibition by 2,3-dioxo-indolines

Felix and Schaefer (1947) reported that 5,7-dinitro-isatin inhibited rat liver enzyme which oxidised p-hydroxy phenylpyruvic acid. They used this inhibition as a test of liver injury produced by 5,7-dinitro isatin and trinitrotoluene, etc.

Bruns (1954) showed that the parent compound isatin also could inhibit enzyme systems. He demonstrated that isatin, alloxan and ninhydrin inhibited milk xanthine oxidase (XDD). He observed that in this respect isatin was a stronger inhibitor of XDD than alloxan and ninhydrin. Isatin (5mM) inhibited the enzyme by 73%, while alloxan
and ninhydrin in the same concentration could inhibit the enzymic activity by only 43 and 31\% respectively. The inhibition was found to be a function of inhibitor concentration as well as of time. In view of the fact that isatin, ninhydrin and alloxan were known to react with amino acids by a Strecker type reaction, the author concluded that the inhibition was the result of the reaction of the inhibitor with the enzyme protein, possibly resulting in deamination and decarboxylation of its N-terminal amino acid. These inhibitors maintained their inhibition properties even when xanthine was substituted by benzaldehyde as substrate.

Shigyo (1957) studied the effect of isatin, homogentisic acid and benzoquinone acetic acid on the degradation of tryptophan to kynurenine by rabbit liver extract. It was found that all the three compounds inhibited the breakdown of tryptophan. Inhibition by isatin and homogentisic acid was overcome by the addition of glutathione, while that by the third compound was not affected.

Calvet and Bozal (1958) studied the inhibition of tryptic digestion by certain drugs including isatin.

Müller (1962) reported that isatin inhibited monoamine oxidase activity in mice liver homogenate. In
a later communication (1965), Müller and Schmiedel showed that administration of isatin, N-methyl isatin, dioxindole and oxindole inhibited monoamine oxidase activity of mice liver homogenates. Isatin-β-hydrazone and isatin-β-imide exerted only an insignificant inhibitory effect. On the other hand, 5-bromo isatin as well as 5-bromo-β-hydrazone were found to be more potent inhibitors in comparison to isatin and isatin-β-hydrazone respectively. After 5 hours of in vivo administration, isatin and 5-bromo-isatin decreased the activity of liver monoamine oxidase by 3 and 60 per cent respectively. These compounds inhibited the brain enzyme only to an inconsequential degree.

Recently Linitskaya and co-workers (1972) demonstrated that isatin thiosemicarbazone activated viral alkaline DNase and inhibited the acid DNase.

(c) Chemotherapeutic activity

(i) Antiviral activity

In 1953 Thomson et al. demonstrated for the first time that isatin thiosemicarbazone (ITSC) was effective against intracerebral vaccinia infection both orally as well as intraperitoneally. This was followed by extensive work on this compound and hundreds of its
derivatives were synthesised by substitution in benzene ring, at N¹ or N⁴ or at all the places simultaneously. The parent compound was subsequently found to be active against rabbit pox, cow pox, Aalastrim, Columbia and variola viruses. Bauer (1955) found this compound to be effective also against vaccinia in mice but not against neurotropic yellow fever, N W S influenza and other viruses. Similarly Bock (1967) demonstrated its inactivity against ectromelia.

Derivates of isatin-3-thiosemicabazone furnish an elegant example of structure-activity relationship. Bauer and Sadler (1960) found that substitution at N¹ enhanced activity against vaccinia virus, but these compounds had no influence on ectromelia virus. On the other hand, substitution at N⁴ yielded derivatives which were active against ectromelia virus but were ineffective against vaccinia virus. N-methyl isatin-β-thiosemicarbazone (Marboran) has been found to be an excellent agent against small pox; it proved highly efficacious during an epidemic.
Although several workers have tried to delineate the mechanism of antiviral activity of 1-methyl isatin thiosemicarbazone, it seems that last word has not been said so far. Furasawa et al. (1966) showed that N-methyl isatin thiosemicarbazone inhibited the viral specific protein synthesis and virus maturation step (assembly of the DNA and the protein coat). In the same year Appleyard et al. showed that N-methyl isatin thiosemicarbazone did not prevent the synthesis of viral DNA. Woodson et al. (1965) added isatin-β-thiosemicarbazone at different times and concentrations to preparations of HeLa cells infected with vaccinia virus. Although no effects were observed up to three hours after infection from this time on, the presence of ITSC resulted in a reduction of the functional half life of viral m-RNA by 15–60 minutes to 5 hours so that synthesis of protein programmed to be translated later than 3 hours was drastically reduced and consequently little (if any) mature viral progeny was formed. Magee and Back (1966) studied the antiviral activity of isatin thiosemicarbazone and its analogues with HeLa cells. ITSC did not affect the early biological and enzymic activities. The N-methyl derivative of ITSC inhibited viral induced DNA synthesis. N-methyl-4', 4'-dimethyl ITSC was much more inhibitory than
ITSC or its N-methyl derivative. Wolfgang et al. (1966) reported that ITSC inhibited the synthesis of early enzymes and arrested vaccinia virus multiplication. Further, it was demonstrated that in the presence of ITSC, certain proteins were altered allosterically and these in turn prevented the functioning of m-RNA.

Besides the above, a myriad of other reports are contained in literature which speak of great potential of isatin-$\beta$-thiosemicarbazone and its derivatives as antiviral agents. It appears that this search for still better derivatives of isatin thiosemicarbazone would continue for many more years to come.

(ii) Anti-tubercular activity

Buu-Hôi and his co-workers (1953) synthesised a large number of hydrazones including isatin-3-hydrazone which were tested for their possible antitubercular activity. Some of the hydrazones were found to be quite potent. Isatin-3-hydrazone, however, exhibited only a weak antitubercular activity. Koshimura et al. (1954) reported that the $\beta$-oxime, semicarbazone, thiosemicarbazone, and phenyl hydrazone also showed some antitubercular activity.

Recently Knotz (1970) reported that acyl hydrazones and semicarbazones of L-isatin acetic acid derivatives were
quite effective antiviral and antimicrobial agents.

(iii) Anti-fungal activity

Claude (1967) reported that N-trichloromethane sulfenyl isatin obtained by the reaction of isatin and trichloromethane sulfenyl chloride possessed potent antifungal properties.

(iv) Anti-Candida albicans activity

Sasaki (1957) tested 44 compounds against Candida albicans in vitro and found 2,2'-dihydroxy-5,5'-dichlorodiphenyl trisulphide to be the most potent compound with little toxicity. Due to the non-availability of the original paper, it cannot be judged whether isatin was also active though it has been referred to in literature.

(v) Anti-brucella activity

Jeney and Zsolnai (1955) tested 183 compounds (including isatin) classified as phenols, nitrophenols, nitroso compounds, quinones, unsaturated ketones, hydroxyamino derivatives, basic compounds, dyes and others for their bacteriostatic action against Brucella abortus in concentrations from 1:5000 to 1:10^6. K, Zn, and Cu methyl-dithiocarbamates, Zn diethylidithiocarbamate and Zn ethyl xanthogenate were also bacteriostatic in concentrations of 1:250,000 to 1:500,000. Due to the non-availability of the original communication, it is difficult for the
present author to judge whether isatin was also found to be active.

(d) **Other potential pharmacodynamic properties**

1. **Neurochemical and CNS affecting activities**

   **Anticonvulsant activity**

   Sareen et al. (1962) found that isatin possessed potent anticonvulsant activity against electroshock seizures in rats. This compound was found to be ineffective against psychomotor and chemoshock seizures induced with metrazol, picrotoxin and strychnine. These investigators had predicted the antiepileptic property of isatin on the basis of the working hypothesis that an excessive accumulation of ammonia in the brain might possibly be one of the causative factors of epileptic seizures, and that isatin by virtue of its structure (the activated 3-keto group) has the capacity to bind free ammonia, and also to cross the blood-brain barrier. They (1962b) further demonstrated that on N-alkylation or acylation isatin lost its anticonvulsant properties. Similarly its reduction to dioxindole and oxindole also offset the antiepileptic activity. Isatin was found to be slightly less potent than Dilantin but the former was much less toxic.

   Müller in the same year (1962), working independently, also reported that isatin antagonised electrical
or pentetrazol tonic seizures in mice. On the contrary, indole was found to be highly toxic and devoid of anti-convulsant activity. Later Müller and Schmiedel (1964) demonstrated that oxindole and dioxindole were quite effective against pentetrazole and electroshock seizures in albino mice. These were, however, found to be highly toxic; the animals died through cessation of breathing.

Recently Kaestner, Müller and Wenzel (1971) found that injection of a single dose of isatin (160 mg/kg) to rats electically implanted with cobalt electrodes caused seizures after 75 minutes of isatin administration. Three out of the seven animals acquired clonic while one animal acquired grand mal seizures which continued for a long time (120 minutes). The electroencephalographic behaviour was used as the detector of the effect of isatin on the epileptogenic focal activity.

Anti-extensor activity

Klingberg and Müller (1968) reported that intraperitoneal administration of isatin to moving rats fitted with chronic electrodes in the visual cortex, lateral corpus geniculatam, dorsal hippo-campus and bulbus olfactorious caused a slight electrocorticogram
desynchronisation with two forms of spindle groups. One form was found in the visual cortex corresponding to photo after-discharges which could be elicited by light flashes. The second form (spindle) was of higher frequency and embraced some portion of the brain, mainly the frontal cortex. Also generalised convulsions were produced although the total muscle was relaxed.

Recently in 1971 several isatin derivatives with the general structure were synthesised. These compounds showed hypotensive, central nervous system depressant and anti-inflammatory activity. One of the compound (R = C₆ H₅) lowered blood pressure of the anaesthetised rats.

![Chemical structure](image)

**Analgesic activity**

Razdan and Razdan (1966) reported that, although isatin itself showed no analgesic property, it did potentiate analgesic action of morphine. Recently Jacques (1970) demonstrated that 5-bromo isatin possessed analgesic and sedative properties against female mice and guinea pigs. The acute toxicity (TD₅₀) of orally
administered 5-bromo isatin for the mouse was 1.75 g/kg. As an analgesic 5-bromo isatin was found to have an edge over aspirin in not showing any side reactions especially lengthening of the blood clotting time.

**Effect on blood pressure and respiration**

Kohli et al.\(^\text{10}\) found that intravenous administration of the drug in doses of 10 mg/kg and 20 mg/kg did not produce any effect on the blood pressure and the respiration both in the dogs and cats. With intravenous doses of 50 mg to 100 mg/kg, there was a slight and transient fall of blood pressure in these animals. The respiration was not affected in these species of animals even with the maximum dose of 100 mg/kg.

**Cathartic action**

Several derivatives of isatin have been found to possess cathartic activity. Bergel\(^\text{83}\) found that phenol isatin, the condensation product of phenol and isatin was endowed with cathartic activity. Later Yamamoto and Kawahara (1937)\(^\text{84}\) found that pentacetylresorcin isatin, diacetyl guaicol isatin, dimetacresol isatin acetate, and diorthocresol isatin all exhibited cathartic activities. Out of these compounds, the last two were found to be quite potent. Recently diacetyl bis (p-hydroxyphenyl) isatin has been found to possess cathartic activity\(^\text{85}\).
Effect on blood sugar

Literature contains conflicting reports on the effect of isatin on the blood sugar level. Hidy (1946) reported that subcutaneous and intraperitoneal administration of isatin to rats in doses ranging from 60 to 200 mg/kg body weight showed no diabetogenic activity. Bruckman and Wertheimer (1947) found that intravenous injection of isatin in doses of 50 to 80 mg/kg in rat produced neither diabetes nor toxicity. Gaede and Frischer (1948) reported that administration of isatin (25 mg/kg body weight) to rabbits by the intravenous route produced at first a temporary slight fall in their blood sugar level, which was followed by a steady rise reaching a maximum of about 170 mg per cent in 4 hours, and then returned to normal level by 24 hours. Only repeated intravenous injection of isatin on alternate days continuously over a period of two months caused cellular damage as evidenced by higher fasting level and greater intolerance to glucose administration. Feeding of 2 gm of glucose to such animals raised the blood sugar level to 300 mg per cent in 4 hours. As no liver and kidney damage was observed in these cases, it seemed that isatin, like alloxan and ninhydrin, might have caused a selective damage or necrosis of the pancreatic beta cells.
Müller and Schultrich (1966) conducted a detailed study on the question of hyperglycemic effect of isatin in order to confirm the previously reported investigations demonstrating its marked hyperglycemic effect. Their findings could not confirm the earlier reports as they failed to observe any significant change in blood sugar level on intraperitoneal administration of isatin to rats, guinea pigs, and rabbits in a dose of 50-200 mg/kg. Estimation of glucose level in blood by different methods showed, if any, only a weak effect of this compound. Oral administration of isatin for 129 days to rats did not affect the increase in body weight of rats. Further, light as well electron microscopy showed no untoward effect of isatin on the animals.

Amin and Singh (1968) observed that oral administration of a single dose of 400 mg of isatin to human volunteers caused a slight (about 10%) but statistically significant fall in their blood sugar level. From these findings the authors concluded that isatin did not seem to mimic the activated keto group activity of alloxan and ninhydrin to any appreciable extent.

Hart and Mcall (1967) demonstrated that oxyphen isatin diminished the absorption of glucose from perfused
rat small intestine. In this respect isatin was not as effective as oxyphen isatin. They further showed that oxyphen isatin prevented the active transport of glucose and not merely the passage of glucose into the mucosal cells.

**Effect on bile metabolism**

Smith and Whipple (1930) reported that though isatin was very slightly effective in increasing the taurocholic acid production in the bile fistula, yet like indene it was quite potent as a cholagogue. Indene and hydrindene dicarboxylic acid had no influence on the taurocholic acid output.

**Anti-sarin poisoning effect**

Isatin-β-oxime along with several other oximes was tested for anti-sarin poisoning effect but it was not found to be effective.

(e) (1) **Plant growth promoting activity**

Thimann (1958) tested some indole derivatives including isatin for their auxin-like activity and found that only indole-3-isobutyric acid showed this property, while isatin and other compounds were found to be inactive. Galstone and Chen (1965), on the other hand, published evidence to show that isatin possessed auxin-like activity. These authors employed a more sensitive technique for their study and demonstrated that isatin as well as oxindole increased the elongation of both etiolated and green pea stem sections. In this respect these two compounds were
as effective as indole acetic acid. Further, introduction of isatin in the reaction medium after maximum elongation had already taken place with indole acetic acid produced no further elongation effect. Besides, isatin helped in the cell division in pith callus tissue of pelargonium. In the light of these observations the authors claimed that they had discovered the first example of an auxin which possessed no carboxyl group. Subsequently, Chen et al. (1966) confirmed the auxin-like activity of isatin in Pisum stem sections in a concentration exceeding 1 mM. However, an aged solution of buffered (phosphate) isatin in a concentration of 0.1 mM was as effective as 1 mM of fresh isatin solution. This interesting observation was attributed to the gradual conversion of isatin to isatic acid. This suggestion was confirmed by using fresh potassium isatate as plant growth regulator when they found that potassium isatate in comparison to isatin was more effective in growth promotion both in sativa coleoptile sections as well as in peas.

James and Wain (1968) investigated the auxin-like activity of isatic acid and 26 derivatives
obtained by substitution in the benzene ring in the wheat cylinder, the pea segment and the pea curvature tests. Isatates substituted in position 3 and 6 manifested diminished activity while the derivatives having substitution in positions 4 and 5 showed enhanced activity in comparison to the parent compound isatate. For example, 4- and 6-bromo-isatates were more active, while 3- and 6-chloroisatates were less active than isatate. Similarly, they examined anthranilic acid as well as some of its derivatives for their plant growth regulator activity. Anthranilic acid itself was inactive while some of its derivatives contained larger groups like iodo or bromo at position 5 were found to be active. Compounds substituted in the 4-position to the carboxyl group were inactive. Several other disubstituted derivatives such as 3,6- and 5,6-dichloro and dibromo were also found to be endowed with auxin-like activity. Wheat coleoptile and pea stem segments metabolized sodium isatate and sodium 5-chloro-anthranilate to an unidentified non-acidic metabolite in each case. The authors further claimed that the activity of these isatates and anthranilates was due to these intact molecules and not due to their metabolites.
Milen and Galstone (1968)\textsuperscript{98} by using 2-$\text{C}^{14}$ labelled isatin demonstrated that it possessed auxin-like activity. They further showed that in plants isatin was mainly converted to anthranilic acid with small amounts of anthranilamide, tryptophan and kynurenine. According to these authors the growth promoting activity of isatin might be due to its conversion to indole acetic acid.

Mitsch (1967)\textsuperscript{99} studied the effect of some growth substances on the induction of flowering of a short-day plant in vitro. Isatin, L-tryptophan and gibberellins prevented the induction of flowers from the buds of Plumbago indica var Angkor, while cytokinins and abscisic acid favoured it.

(ii) Herbicidal action

Isatin and some of its halogen derivatives, i.e. 5,7-dichloro, 4-chloro, 6-chloro and 5-bromo isatin were found to be quite effective herbicidal agents for killing dicolytedonous plants but not grasses\textsuperscript{100}.

B. Metabolism of 2,3-dioxindolines

Anabolism

Böhm (1940)\textsuperscript{101} demonstrated that ingestion of o-nitro phenylglyoxylic acid by rabbits resulted in the excretion of isatin in urine. This seems to be the first
observation on isatin biosynthesis. However, o-nitro mandelic acid yielded dioxindole which was converted to isatin by air. Ichihara (1956)\textsuperscript{102} studied the bacterial decomposition of indoles and found that the parent compound could be converted to isatin which was subsequently metabolised to anthranilic acid.

Satoshi Matsumoto (1958)\textsuperscript{103} isolated and examined several compounds from the urine of cancer patients and using counter-current distribution and chromatographic techniques he was able to identify these as dihydroxyindole, isatin and indirubin. Subsequently Maki (1959)\textsuperscript{104} also confirmed the presence of isatin along with indigo and indirubin in the urine of cancer patients.

Tahata (1959)\textsuperscript{105} showed that perfusion of rabbit liver with blood containing indole gave rise to isatin, indican, anthranilic acid and urochrome. King and his co-workers (1966)\textsuperscript{106} observed that rats fed with indole were found to contain 5 to 8 per cent isatin in their urine.

Beever and French (1954)\textsuperscript{107} demonstrated that crude extract from several plants oxidised N-acetyl indoxyl. They tentatively identified the product as N-acetyl isatate.
Recently Reimann and Javet (1967) isolated 5-chloro-6-methoxy-1-methyl isatin from Mivonommospore carbonaceae.

**Catabolism**

As early as 1903 Hilderbrandt and Kleist independently observed that isatin in animal body was degraded to anthranilic acid. Ichihara et al. (1966) confirmed the above findings when they showed that enzymes present in rabbit liver as well as in bacteria converted isatin to anthranilic acid.

Bohm (1941) demonstrated that when isatin-\(\beta\)-chloride or isatin-\(\beta\)-oxime was administered to rabbits, excretion of ester in the urine was increased while that of indican was not affected. However, in the case of isatins no glucuronate conjugation was observed.

Chen et al. (1966) published evidence to show that isatin in plants was metabolised to isatate, and recently Milen and Galstone (1968) noted that isatin was mainly converted to anthranilate in pea stems (loc. cit). Plant enzymes have also been reported to act on isatin. Beevers and French (1964) found that an enzyme in a
crude extract from several plants converted N-acetyl isatin to N-acetyl isatate.

C. Other physiochemical properties in relation to biological activity

The absorption spectra of isatin had attracted the attention of investigators since the last century and they used it to prove the existence of keto-enol tautomeration in organic compounds. Sadler et al. (1959) studied the infrared spectra of isatin and several of its derivatives and showed that a relationship existed between the infrared spectra and L-amino acid dehydrogenase activity of these compounds. Several workers have established the structure of complexes formed by the interaction of isatin-3-oxime with cations by the examination of their ultraviolet spectra. Chen et al. (1966) with the help of ultraviolet spectrum demonstrated that auxinlike activity of isatin was due to its conversion to isatate.

Cassebaum (1954) determined the oxidation-reduction potential of isatin and several of its derivatives and demonstrated that the L-amino acid dehydrogenase activity of these compounds could be related to their oxidation-reduction potentials.
Sumpter et al. (1949) for the first time studied the polarographic behaviour of isatin at different pH and demonstrated the cleavage of isatin molecule at higher pH. Later Korshunov and Schennikova (1950) also polarographically confirmed the findings of Sumpter et al. Polarography of substituted isatins has also been studied.

D. Analytical uses

1. Estimation of organic compounds and metals

Sawicki et al. (1959) found that isatin chloride reacted with phenols and polynuclear hydrocarbons to give cationic dyes which were highly coloured. The intensity of the colour was proportional to the amount of phenols or hydrocarbons. Nakagawa and Namata (1970) estimated small amounts of thiophene in benzene with the help of isatin. Isatin reacts with thiophene in the presence of an oxidising agent to form an intensely coloured indophenine which is measured at 550-590 nm. Knotz (1970) found 1-isothiocyanatomethylisatin to be a useful reagent for the detection and estimation of primary and secondary amines. Recently Fouad (1971) used isatin for the colorimetric determination of proline in serum and protein hydrolysates.

Isatin-3-oxime has been extensively employed for the detection as well as estimation of metals. Hovorka and
Sykora (1938) were the first to report that isatin-3-oxime forms coloured precipitates with metals like Ag, Pb, Cu, Hg, Fe, Ni, Co and Ti. Report of these authors led to the publication of numerous other reports demonstrating the ability of isatin-3-oxime to form coloured complexes with cations which has also been used for the quantitative estimation of some of these ions. Two important examples are: (1) Buscarons and Izquierdo (1964) used N-ethyl isatin-3-oxime for the gravimetric estimation of uranium; (ii) Divis (1969) estimated Cu** with isatin-3-oxime. The author was able to determine very small amounts (0.05 to 3.13 µg/ml of Cu** by this method. It seems that use of isatin-3-oxime or its derivatives for the estimation of cations would continue for a long time.

Isatin 3-thiosemicarbazone has been used for the detection of metals, while recently Taha and Khatab (1971) found that isatin-3-phenyl hydrazone also formed chelates with Zn**+, Mg**+ and other cations.

(ii) Detection of amino acids by chromatography

Acher et al. (1950) by using paper chromatography have shown that isatin in n-butanol-acetic acid medium was highly specific for proline and hydroxyproline which
gave intensely blue-green colours with isatin. They based this work on the earlier finding of Grassmann and Arnim (1934) that proline and hydroxyproline gave coloured products with isatin.

This finding led to publication of a sizable amount of data in which isatin in preference to ninhydrin has been used as chromogenic reagent for the detection and estimation of amino acids using different types of chromatography. For example, Smith (1953) employed isatin as a spraying agent for amino acids in chromatography. Instead of the usual technique of spraying a colouring agent on the dried chromatogram, he used isatin along with ninhydrin and diemethyl-aminobenzaldehyde as a dipping agent for the detection of amino acids by paper chromatography. Subsequently, Saifer and Oreskes (1954, 1956) found that isatin was most useful for the qualitative identification of amino acids with circular paper chromatographic technique. Further, these authors demonstrated that the following amino acids gave no appreciable colour with isatin when the amount of these amino acids was less than 10 µg: glycine, alanine, valine, leucine, isoleucine, serine, threonine, glutamine and
asparagine. Likewise, McKee and Urbach (1953)\textsuperscript{129} and Boyarkin (1958)\textsuperscript{130} have also made use of isatin as a colouring agent for the identification of amino acids. Bonomi and Vecchioni (1969)\textsuperscript{131} have found isatin to be an excellent spraying agent for the chromatography of amino acids in general and for proline and hydroxyproline in particular. Krawczyk (1962)\textsuperscript{132} made an interesting use of isatin when he differentiated peptides obtained by tryptic digests of human and horse haemoglobins. He showed that peptides obtained from human haemoglobins were mostly stained pink while those from horse haemoglobins stained dark violet or violet pink on treatment with isatin. He has ascribed this difference to possibly different amino acid composition of human and horse haemoglobins. Morozova (1965)\textsuperscript{133} published evidence to show that isatin when used in collaboration with a metal salt was much superior as a reagent for detection of amino acids by chromatography as compared to when isatin was used alone. Mixing of a metal salt with isatin considerably raised selectivity of isatin reaction with amino acids as well as it increased greatly the visibility of amino acids spots. A few other reports also show that isatin can be used as a spraying agent for the detection of amino acids\textsuperscript{134-136}. 