

BLUE ADSORPTION COMPOUNDS

OF IODINE

by

FREDERICK JOHN EATON.

Thesis for the Degree of Ph.D.

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QUALIFICATIONS AND EXPERIENCE.

The primary schooling of the candidate was received at Arbroath High School and later at Craigie School, Perth. From this latter school in 1912 a Duncan Bursary was obtained by examination. Three years were then spent at Perth Academy, the Lower Leaving Certificate being obtained in 1916. The year 1916-1917 was spent in the Buying Dept. of Thos. Nelson and Sons, Publishers. In October 1917 a course of Chemistry was entered upon at the Heriot Watt College, Edinburgh. Having passed the entrance examination of the University of Edinburgh, and after a short period of military service with the University Battery O.T.C., the candidate continued the Heriot Watt College course of Chemistry and began the B.Sc. (pure science) course of the University of Edinburgh. At the end of the first year the 1st B.Sc. examination was passed in Mathematics (2nd Ordinary), Natural Philosophy and Chemistry. In 1920 the second examination was passed in Geology, Intermediate Natural Philosophy and Intermediate Chemistry. The degree of B.Sc. was obtained in 1921.

Since then the candidate has been occupied in post-graduate research work in the Department of Medical Chemistry, University of Edinburgh, under the guidance of Prof. Barger.

In/

In 1923 an application was made to the Institute of Chemistry, of which the candidate is a member, for the degree of A.I.C. which was granted.

HISTORICAL PART.

The phenomenon presented by the product of the action of iodine on starch, the so-called "starch-iodide", has been the subject of much discussion and not a little controversy. In a communication by Barger and Starling, J.C.S. T.107, 411 (1915), attention was directed to the fact that in addition to starch, cholalic acid and saponarin there exist a number of natural and synthetic substances which give blue additive compounds with iodine. These compounds are here grouped under the name "Blue adsorption compounds of iodine".

With the exception of the work of Mylius and Küster on cholalic acid and Barger and Field on saponarin, all the research on the blue adsorption compounds of iodine has been confined to "starch-iodide". "Starch-iodide" was discovered, it is supposed, by Stromeyer in 1813, Thorpes Dict. Vol. III, page 565, and although much work has been done there appears to be very little concordant opinion as to the real nature of "starch-iodide". The conclusions arrived at seem to depend on the method and line of attack. Thus Pohl, Journ. für prakt. Chem. 83, 35 (1861), considered that "starch-iodide" was not a true chemical compound since it was readily decomposed/

decomposed by water. Duroy, Compt. Rendus 51, 1031 (1861), went further by adding that the iodine was attached to the starch by a type of capillary affinity, thus accounting for the two principal properties of "starch-iodide", namely, decomposition by water and decolorisation by heat. On the other hand Guichard, Bull. Soc. Chim. (1) 5, 115, 278 (1863), from consideration of the action of heat, silver nitrate and potassium iodide on the blue compound, considered it a combination or mixture of combinations in definite proportions of starch and iodine. Boudonneau, Compt. Rendus. 85, 671 (1877) also Bull. Soc. Chem. (2) 28, 452 (1877), was of the same opinion and estimated the iodine content of starch iodide deducing therefrom the formula $(C_{12}H_{10}O_{10})_5 I$. In passing it is curious to note the old formula for starch given by Boudonneau which was continued in France much longer than elsewhere. Duclaux, Ann. Chim. Phys. 4th, 25, 264 (1872), gives a résumé of previous work and was led to support a theory of molecular adhesion, the phenomenon observed being analogous to that of lead and charcoal or otherwise "starch-iodide" was an example of real solution, the term solution being used in its widest sense. Thus according to the line of attack "starch-iodide" is supposed to be either a definite chemical compound or that the iodine is merely lightly attached to the starch in no fixed proportion./

proportion.

A systematic investigation of the iodine content of "starch-iodide" is to be found in a series of papers by Rouvier, Compt. Rendus. (1892) 114, 128, 749, 1366; (1893) 117, 281, 461; (1894) 118, 743, and (1897) 124, 565. Rouvier considers that different compounds are formed according to the conditions of the reaction. If in the reaction mixture starch is present in excess, what Rouvier called the inferior limit, the formula corresponded to $(C_6H_{10}O_5)_8 I$, when iodine is in excess, superior limit, a series of compounds are formed corresponding to $(C_6H_{10}O_5)_{16} I_2$, $(C_6H_{10}O_5)_{16} I_3$, $(C_6H_{10}O_5)_{16} I_4$ and $(C_6H_{10}O_5)_{16} I_5$. Rouvier concluded that the system, i.e. starch, iodine "probably obeys the law of Berthelot and Jungfleisch relative to the partition of a body dissolved between two immiscible solvents which, other conditions being the same, is proportional to the concentration of iodine in solution." The partition coefficients were calculated and were found to be in close agreement with the experimental figures. In direct contrast are the results of Andrews and Goettsch, J. Am. Soc. (1902) 865, who state "Solutions of "starch-iodide" on shaking with chloroform yield at first considerable amounts of iodine to the latter but after removal of the first portion give up iodine very slowly/

slowly, showing no indication of a distribution coefficient of iodine between starch and chloroform as might be expected if the iodine were merely dissolved in the starch." Also more recently Padova, Atti. R. Accad. Lincei (1908) V, 17, I. 214-215, states.- "Conclusions drawn from the coefficient of distribution between solutions of iodine starch and carbon tetra chloride would be valid only if the phase law were applicable, which is not the case. There is no authority for considering a suspension either as a single phase or as composed of two phases, neither can it be admitted that amorphous compounds or substances in the form of minute suspended particles are capable of forming solutions which are saturated in the sense in which the term is applied to solutions of crystalline substances." Rouvier also found that if insufficient excess of iodine was present to give rise to the compound $(C_6H_{10}O_5)_{16}I_5$ two phenomena are apparent. Firstly, fixation of the iodine on the starch in definite proportions and secondly, fixation of the remaining iodine in the dissolved state. In other words, if starch paste is treated with excess of iodine but not sufficient to give rise to the compound $(C_6H_{10}O_5)_{16}I_5$, the quantity of iodine fixed increases with the quantity of iodine added.

Andrews and Goettsch, J. Am. Soc. (1902) 865, in support/

support of the definite compound theory, bring forward the following arguments.-

(1) Starch and iodine cannot unite to produce the blue substance in the absence of water, a phenomenon frequently characteristic of chemical combination but not of solution in organic solvents." This has also been observed by Zander, Pfluger's Archiv. (1897) 66, 545-573, and Stocks, C.N. (1897) 56, 212.

(2) "The temporary decolorisation of the blue compound by heat is much better explained as a phenomena of dissociation than of solution." The contrary opinion is held by Bückner, Monat. Chem. 4, 889-906 (1883).

(3) "The fact that in the system starch, water, chloroform and iodine a rise in temperature has a very powerful effect in causing iodine to pass from the starch to the chloroform, indicates that the union of starch and iodine is exothermic and agrees very well with the hypothesis of a dissociable compound."

(4) "The fact that the amount of iodine taken up by the starch under different conditions as to the temperatures to which starch is subjected either before or after iodine is added is naturally explained by the view that the molecule undergoes depolymerisation by steps, giving rise to two or a series of starch iodides of different composition. This also explains why no dissociation constant can be deduced.

(5)/

(5) "The remarkably low vapour tension of the iodine in starch, while quite natural if the iodine is considered as combined with the starch, is exceptional and difficult to understand on any other basis."

The authors deduced two formulae.-

The one corresponding to the iodide obtained by treating clear starch made at a temperature of 150° with iodine at ordinary temperatures $(C_6H_{10}O_5)_{12} I$.

The other corresponding to iodide obtained by treating starch with excess of iodine at a temperature of 100° for a short time $(C_6H_{10}O_5)_{12} I_2$. In conclusion these authors consider "starch-iodide" as a dissociable additive compound of iodine with starch molecules more or less depolymerised according to the temperature employed and subject to variation in the ratio of starch to iodine.

More recently Eüler and Myrbäck, A (1922) 428, 1-24, have obtained staircase adsorption curves with steps corresponding to two compounds $(C_6H_{10}O_5)_{12} I_2$ and $(C_6H_{10}O_5)_{12} I_4$ with soluble starch, and $(C_6H_{10}O_5)_{18} I_2$ and $(C_6H_{10}O_5)_{18} I_4$ with potatoe starch (Arkiv. Kem. Min. Geol. (1922) 8. No.9, 1-29.

In contrast to the above papers are the results arrived at by Küster, A (1894) 283, 360-379, who after a large series of experiments in which an attempt was made to find a ratio between iodine in starch and iodine in solvent was led to the belief that "Blue iodide of starch/

starch is to be regarded as a well-defined solution of iodine in starch."

One great difficulty confronting the investigator of "starch-iodide" is the analysis of its iodine content. Owing to its instability to water and organic solvents and hence the difficulty of getting it in a pure state, renders it necessary to estimate the iodine by indirect methods. Such methods include shaking with organic solvents such as benzene or toluene as used by Euler and Myrbäck (loc. cit.) or more usually the estimation of iodine before and after adsorption by sodium tetrathionate, the "starch-iodide" being precipitated out by ammonium sulphate and filtered off before the final estimation of iodine Küster (loc. cit.). Numerous physical-chemical methods have been used with varying success. Musset, Chem. Central (1896) II, 611, from experiments on dialysis deduced that the iodide was a definite compound. Friedenthal, Centralblatt (1899) I, 1161-1162, used depression of freezing point without obtaining any definite results. Katayama, Zeitsch Anorg. Chem. (1908) 56, 209-217, making use of a tintometric method considered "starch-iodide" as a solution of iodine in starch. This was criticised by Padoa, Atti. R. Accad. Lincei (1908) V, 17, I, 214-215, who contended that "colorimetric measurements give results of doubtful value when applied to suspensions the size of whose particles may/

may influence both the quality and quantity of the coloration." Rodewal and Kattein, Zeitsch. physikal Chem. (1900) 33, 586, measured osmotic pressure of the starch iodide without results of a definite character.

The part played by salts, and in particular by potassium iodide in producing or assisting to produce the blue "starch-iodide", has given rise to a large number of papers. The stabilising effect of potassium iodide has been long known and was first observed by Payen, Compt. Rendus 61, 512 (1865), who showed likewise that the presence of the chlorides of sodium or potassium increased the sensitivity of the reaction.

This has since been confirmed by:-

- (1) Meineke, Chem. Zeit. (1894) 18, 157-160, who showed that the presence of hydriodic acid or iodides was not specific but that the blue colour results in presence of electrolytes such as, the chlorides of K, Na, NH_4 , Ca and Ba; the sulphates of K, Na, NH_4 and Mg; potassium alum and the bborates of K and Na.
- (2) Lonnes, Zeit. Anal. Chem. 35, 409-436 (1894), who showed that the presence of small quantities of iodides increase the sensitivity.
- (3) Barger and Field, J.C.S. T. (1912) 101, 1402, in the case of saponarin observed increased sensitivity/

sensitivity in presence of divalent and trivalent cations such as barium chloride and lanthanum chloride.

(4) Kolthoff, Pharm. Weekblad (1919) 56, 391-404, who found that the presence of salts, especially potassium iodide and of acids, increased the sensitivity.

and (5) Firth and Watson, J. Soc. Chem. Ind. (1923) T. 42, 308-310, stated that in a system, iodine, starch and chloroform, the amount of iodine transferred to the starch depends on the amount of potassium iodide present. The iodine added being proportional to the concentration of potassium iodide up to a concentration of 0.192 gms. per litre, further additions increase the amount transferred but not in proportion with the amount added.

Mylius B (1887) 688, deduced a formula for "starch-iodide" which contains hydriodic acid or potassium iodide $[(C_6H_{10}O_5)_n I]_4 HI$. This paper provoked much discussion, Mylius' views and the resulting discussion may be stated as follows.-

Mylius:- Iodine solutions which colour starch blue, contain hydriodic acid or one of its salts.

This is denied by Stocks C.N. (1887) 56, 212 and (1888) 57, 183, Berezeller, Biochem. Zeitsch. (1917) 84, /

84, 106-107 and Euler and Bergman, Kolloid Z. (1922) 31, 81-89, who state that with perfectly pure starch and pure iodine the blue colour still results. On the other hand Barger and Field (loc. cit.) found that a solution of iodine in water free from iodide does not colour starch or cholalic acid or saponarin.

Mylius:- The presence of substances which destroy hydriodic acid hinder the production of the starch iodide. Silver solutions decolorise starch iodide. This Mylius explains as follows:- The silver nitrate withdraws the hydriodic acid from the iodide of starch, giving rise to silver iodide and free acid, at the same time starch and iodine are set free. The iodine liberated acts on the silver nitrate, giving silver iodide and iodic acid. The addition of hydriodic acid liberates free iodine and the conditions for the formation of starch iodide are once more established. The probable presence of a soluble double compound of silver iodide explains the phenomenon observed by Guichard, Bull. Soc. Chim. (1863) (1) 5, 115, 278, that no silver iodide is precipitated. Stocks, C.N. 57, 183, (1888), does not agree with this but does not state his case very clearly.

The results of Mylius have been confirmed by Padoa and Savaré (1905) (V) 14, I, 467-476, who arrived at their decision from experiments on the conductivity of/

of iodide of starch and showed that it must be considered as an additive product of iodine, starch with hydriodic acid or potassium iodide, the molecular ratio being as $(C_6H_{10}O_5)$ is to 4 and by Hale, J. Am. Soc. (1902) 28, 438.

In addition to the blue "starch-iodide" Mylius B. (1895) 385, drew attention to a brown iodine rich compound. This substance can be produced by acting on starch paste with iodine dissolved in a solution of zinc or better, cadmium iodide. It is supposed that the resulting colour depends on the degree of ionisation of the solvent. Another explanation put forward by Andrews and Goettsch, J. Am. Soc. (1902) 865, is based on the supposition that iodine acts as a weak base. This accounts for the fact that when potassium acid carbonate or other weakly alkaline solution is added to "starch-iodide" which has been rendered brown by the addition of iodic acid, the blue colour is restored. The iodic acid removes the iodine from the "starch-iodide" in the form of iodine and iodate and on addition of a stronger base the weaker one (iodine) is displaced and placed once more at the disposal of the starch with the above result.

It has been shown by Jakovkin (1894), Zeit Ph. Chem. 13, 539, and confirmed by Bray and MacKay, J. Am. Soc. (1910) 32, 914-932, and by Jones and Hartmann, J. Am. Soc. (1915) 37, 241-258, that in a solution/

solution of iodine in potassium iodide there exist KI_3 molecules. These molecules give a characteristic adsorption spectra which is destroyed by the addition of starch. Tinkler, J.C.S. T. (1907) 91, 996. Since the disappearance of the KI_3 molecules synchronises with the formation of the blue colour, it might be argued that "starch-iodide" is a periodide of starch. Periodides of benzamide and certain anilides have been obtained by Moore and Thomas, J. Am. Chem. Soc. (1914) 36, 1928, some of which admit of purification and consequently unlike "starch-iodide" of accurate analysis. For example, besides other similar properties the formula deduced for benzamide periodide $(C_6H_5CO NH_2)_2 I_2 HI$ bears a close resemblance to the formula for "starch-iodide" deduced by Mylius.

By some investigators it has been thought that starch acts towards iodine in the nature of a protective colloid.

Amann, Zeitsch. Chem. Ind. Kolloide (1910) 6, 235, has shown that certain solutions of iodine in organic solvents can be resolved under the ultra-microscope. In petrol ether a violet coloured solution results which contains numerous particles visible under the ultra-microscope. Harrison, Zeit. Chemie Kolloide (1911) 9, 5, claims to have obtained a solution of colloidal/

colloidal iodine which is unstable unless protected by some means. In more recent years Bordier and Roy, Compt Rendus (1916) 163, 567-569, state that the brown solution of iodine in water exhibits Brownian movement and is a pseudo-solution of iodine in water capable of being stabilised by the addition of gelatine solution.

Attention has been drawn to the very great part played by the physical condition of the substance at the moment of adding iodine. An excellent example is afforded by cholalic acid which gives from alcoholic solution with iodine a blue additive crystalline compound supposed by Mylius to have the formula $(C_{24}H_{10}O_5)_4 I_4 HI + nH_2O$ and by Küster, Physikal Chemie (1895) 16, 156-163, as neither a solid solution nor a true chemical compound but a crystal structure analogous to that which the acid forms with water alcohol etc. If the cholalic acid is dissolved in alkali and precipitated in presence of iodine in potassium iodide by acids, amorphous adsorption takes place, the amount of iodine added being much larger than in the case of the crystalline additive compound. See diagram Barger and Field (loc. cit. p. 1407). Saponarin has been found by the same authors to give a true adsorption curve which corresponds to the formula deduced by Freundlich $x/m = a c^{1/n}$. Moreover that the adsorbent should be in the colloidal state seems to be an essential condition for the production of/

of the blue colour. Saponarin, for example, is a negative colloid and its iodide is susceptible of precipitation by cations in agreement with Schultz's law. Further examples of this are to be found in a paper by Barger and Starling, J.C.S. T. (1915) 107, 411. In this paper are given a list of substances which act as adsorbents to iodine. These comprise quinones, phthalides, pyrones, thio flavones, etc. It has been noted that all these substances possess what is termed across-conjugated double linkage. These facts together with the rapidity with which equilibrium is reached in the system starch iodine solvent have led some investigators to attribute the phenomena to adsorption.

Starch-iodide is by no means an isolated example of this curious class of compounds. Disseminated through chemical literature are examples of substances which can be made to give blue additive compounds with iodine. Such are basic lanthanum acetate. Biltz, B. (1904) 37, 719, also attributed to Damour (see Duclaux, Compt. Rendus 74, 533 (1872), isolichenin Brown, Am. J. Physiol. (1898) I, 455-460, narceine, Stein, Fr. 9, 380 and Pelletier A 16, 48, derivatives of euxanthic acid, Gräbe B 33, 3360 (1900) and the cystine form of ergothioneine. Barger and Ewins, J.C.S. T. (1911) 99, 2338, see also Tranet, J. Pharm. Chim./

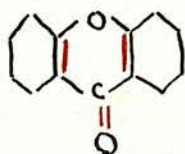
Chim. (1909) (VI), 30, 145. A large number of these substances, such as the esters of euxanthic acid, have been discovered by accident and in most cases the similarity of the reaction and properties to those of starch-iodide has not been noted.

It is obvious, as has been observed by Freundlich, Kapillar-chemie 1922 ed. page 255, that since on the addition of starch to a brown solution of iodine that solution turns blue, some chemical action must have taken place but the reason for this change is still obscure.

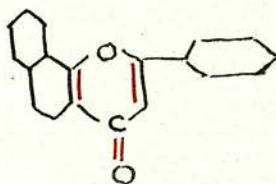
EXPERIMENTAL PART.

RELATION OF CHEMICAL CONSTITUTION TO
ADSORPTIVE POWER.

It has been observed by Barger and Starling, J.C.S.T. 107, 415 (1915) that most substances which are known to add iodine, comprising quinones, pyrones, flavones etc. possess a cross-conjugated double linkage. For example Xanthone I and α -naphtha flavone II.



I

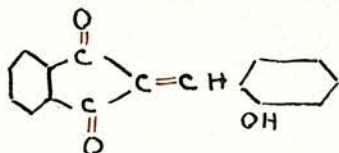


II

It thus seemed probable that there existed some connection between this linkage and adsorption of iodine. To test this, several diketohydrindene derivatives, which were known to possess this linkage but which were not known to add iodine, were prepared. In addition these substances all possess a fairly large molecular weight and, with the exception of the sodium salt of diketohydrindene carboxylic ester, are insoluble in water. They were thus susceptible of precipitation out of solution in presence of iodine, a condition which in previous cases has been found to be/

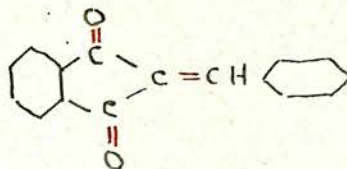
be essential for the production of an additive compound.

ORTHO-HYDROXY-BENZYLIDENE-INDANEDIONE



Kostanecki and Laczkowski B. 30, 2138 (1897), could not be made to add iodine.

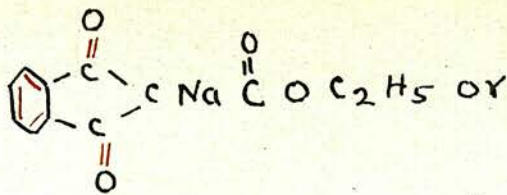
BENZYLIDENE-INDANEDIONE



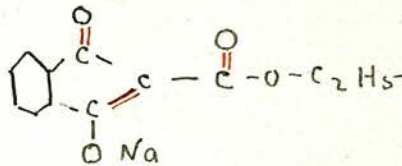
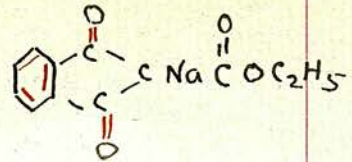
Nathanson, B 26, 2578. On addition of iodine this substance immediately crystallises out without addition of iodine.

SODIUM SALT DIKETOHYDRINDENE CARBOXYLIC ESTER.

Wislicenus and Kötze A 252, 72. In contrast to the above compounds the system of cross-conjugated double bonds in this substance is only apparent when one takes into account the double bonds of the benzene ring as in I, or on the supposition that the sodium atom wanders as in II, gives the reaction.



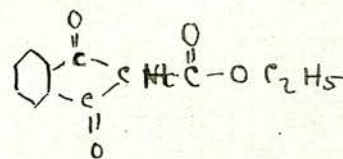
I



II

By dissolving in water and adding a solution of iodine in potassium iodide, the limit concentration of iodine was found to be $N/200$. If alcohol is used as solvent, the substance remains in true solution and so does not give the blue colour. On warming, the blue precipitate dissolves and separates out again on cooling. In connection with this substance there exists an interesting paper by Liebermann and Flatow B 33, 2433, who, after treating a boiling aqueous solution with powdered iodine, succeeded in isolating two products, one of which was white and insoluble, the other green and soluble, in chloroform. The authors did not determine the constitution of the green compound.

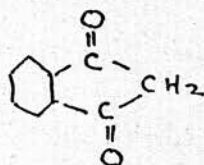
DIKETOHYDRINDENE CARBOXYLIC ESTER.



Wislicenus, B. 20, 593, although it possibly possesses
a/

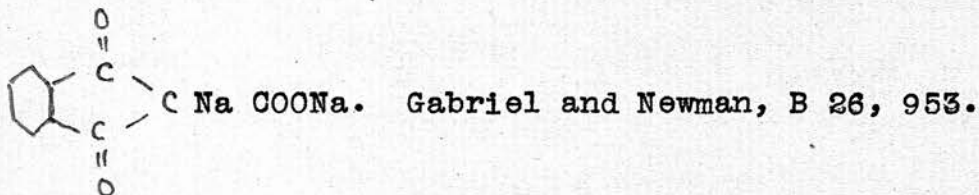
a similar system of bonds as its sodium salt gives a brown precipitate with iodine.

DIKETOHYDRINDENE.



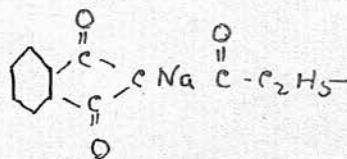
Kaufmann B 30, 385, on being tested gave a brown precipitate.

DISODIUM SALT OF α - γ -DIKETOHYDRINDENE CARBOXYLIC ACID.



gives the reaction at an iodine concentration of N/100. The above results make it probable that if any system of double bonds does exist in the sodium salt, they must be represented by the formula II. This was further tested by the preparation of propionyl diketo hydrindene of which the sodium salt is easily obtained.

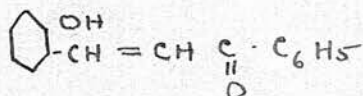
PROPIONYL DIKETOHYDRINDENE



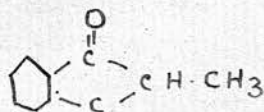
Ernest Schwerin, B 27, 104 (1894). Neither this substance nor its sodium salt could be made to add iodine. The difference between this substance and the sodium/

sodium salt of diketo hydrindene carboxylic ester, is in the end grouping. The former, having a keto-grouping while the latter, has an ester grouping, the rest of the molecule being the same. Also if the one gives an enol form it might be expected that the other would do likewise. Thus it appears probable that a formation of a blue additive compound with iodine is not dependent on a specific system of double-bonds, or if dependent, is affected markedly by the addition of certain groups. This point has been examined and will be dealt with in the next section.

ORTHO-HYDROXY BENZYLIDENE ACETOPHENONE



Borsche, B 33, 1327, an improved method on that of Bablich and Kostanecki, B 29, 233. This substance also, although unsaturated, does not apparently contain a cross-conjugated system of bonds yet it gives the iodine reaction. From alcohol a limit of N/100 is obtained while from alkali by means of acidified iodine solution a limit of N/2,500 is obtained. Contrary to what might be expected, its benzoyl derivative (Harris and Busse, B 29, 379) does not give a blue colour.

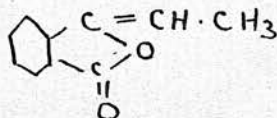
METHYL α - γ -DIKETOHYDRINDENE

Nathanson B 26, 2581. This substance is inactive, thus further demonstrating the marked effect of different groups on adsorptive power.

REACTION OF CERTAIN PHTHALIDES TO IODINE.

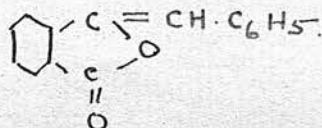
Methyl α - γ -diketohydrindene is most conveniently prepared through the phthalide which is easily transformed by heating with sodium methoxide to the sodium salt of methyl α - γ -diketohydrindene. This phthalide was found to add iodine with difficulty, thus adding a new class of compounds in which this peculiar reaction is to be found.

ETHYLENE PHTHALIDE



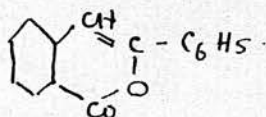
Gabriel B 19, 939 (1886). On adding an alcoholic solution to N/50 iodine, a brownish opaque pseudo-solution is obtained which gradually on standing gives rise to a greenish blue amorphous compound which in turn slowly changes back to brown.

BENZYLIDENE PHTHALIDE



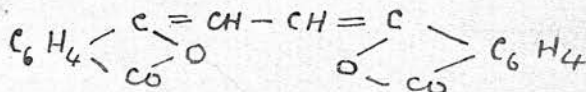
Gabriel, B 18, 3470 (1885), is very reactive, showing the influence exerted by the substitution of the benzene ring for the methyl group. An alcoholic solution containing only 0.004 milli mols gives a blue colour at N/100 and from a solution in alcohol and glacial acetic acid the reaction is possible at N/500. Unlike the substances previously mentioned, this substance gives a colloidal solution which remains stable for several hours and which, on addition of iodine is not precipitated out immediately but remains like starch in pseudo solution.

3-PHENYL ISO-COUMARIN



Gabriel, B 18, 1251 and 3471, can be obtained from benzylidene phthalide by an interesting series of reactions. This was prepared and tested since it has previously been found that most coumarins give the iodine reaction. This iso-coumarin was found to add iodine but only when concentrated N/5 iodine was used.

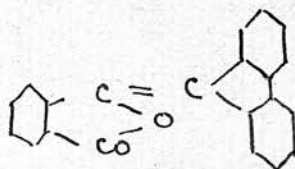
ETHYLENE DIPHALIDE.



Kaufmann/

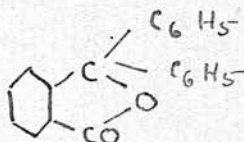
Kaufmann, B 30, 386 (1897), is very insoluble in alcohol, more so in glacial acetic acid but in every case it separated out without addition of iodine.

FLUORENE PHTHALIDE



Wislicenus and Neber, A (1919) 418, 274-283, is found to be negative

DIPHENYL PHTHALIDE



Friedel and Crafts, A de Chemi et de Phys. (6) I, 523, (1884) see also Baeyer, A 202, 50 (1880) in which the double bond has been saturated, gives, as would be expected, a negative reaction.

From the results so far investigated, it appears certain that only unsaturated compounds are reactive. This is in agreement with the results obtained by Barger and Starling who observed that flavones with a reduced pyrone ring are indifferent to iodine. This residual valency cannot be attributed to any particular grouping of double-bonds such as exist in a cross-conjugated system, since some substances possessing this grouping (ortho hydroxy benzylidene indanedione, benzylidene indanedione) do not add iodine while others (sodium/

(sodium salt diketo hydrindene, benzylidene phthalide etc.) in which the group is either absent or obscure, give a positive result. The most reactive substances are capable of giving colloidal solutions more or less unstable and the state of division in which the substance is precipitated in presence of iodine plays a very large part in determining whether or not that substance will add iodine. On the other hand all lyophobic colloidal solutions do not have the property of giving a blue colour with iodine, gum benzoin being found to be indifferent. Attention is directed to the difficulty with which some substances add iodine; ethylene phthalide can be made to add iodine only under certain very limited conditions. It is thus all the more probable that this peculiar type of unstable additive compound is far more frequent than is at present known, and that other examples of substances which give this reaction will only be forthcoming in sufficient numbers to render the solution of this problem a possibility when the iodine reaction is tried as a matter of course by investigators.

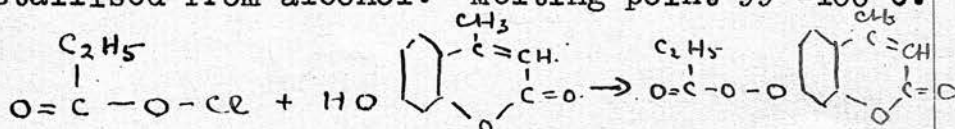
EFFECT OF CERTAIN GROUPS ON ADSORPTIVE POWER.

It has been found that the sensitivity of substances containing hydroxyl groups is greatly increased by the formation of the corresponding benzoyl deriva- /

derivative. 7-hydroxy - 4-methyl coumarin (β -methyl umbelliferone) yields the reaction only in concentrated solutions from alkali, being too soluble in alcohol, whereas its benzoyl derivative is extraordinarily reactive, a 0.1% solution giving immediately dark blue at N/1000. On the contrary the acetyl derivative cannot be made to add iodine. In this section, certain other derivatives have been prepared and their reaction with iodine will be dealt with.

ETHYLCHLORCARBONATE DERIVATIVE.

β -methyl umbelliferone was dissolved in normal NaOH solution, the solution cooled in a freezing mixture of ice and salt and the calculated quantity of ethyl chlorcarbonate added drop by drop. The temperature of the reaction mixture was not allowed to rise beyond 5°C. The ethylchlorcarbonate derivative separates out. It was filtered at the pump and recrystallised from alcohol. Melting point 99°-100°C.



ANALYSIS:-

Weight of substance taken = 0.2658 grams.

Wt of CO₂ = 0.6091 grams. Wt of H₂O = 0.1171 grams.

Calculated for C ₁₃ H ₁₂ O ₅	C = 62.9%	H = 4.84%
Found	C = 62.5%	H = 4.9%

REACTION TO IODINE.

A saturated solution of this substance in alcohol, 0.67%, gives blue at once at N/40. With N/80 the blue colour is obtained, but gradually fades, the substance crystallising out colourless.

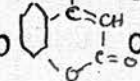
PHENYLACETIC ACID DERIVATIVE.

This was prepared by condensing phenylacetic acid chloride with β -methyl umbelliferone by Einhorn's pyridine method. The acid chloride was prepared after Vanino and Ohrde, B 29, 1727, see also Anschutz and Berns, B 20, 1389, by heating the acid with PCl_5 . The acid chloride was purified by distillation in vacuo B.P. $104-105^\circ\text{C}$. at 23 mm. The reaction mixture was poured into a large volume of water acidified with hydrochloric acid. A solid separated out, which was filtered off, washed with water to remove adherent pyridine and recrystallised from dilute alcohol. M.P. $102-103^\circ\text{C}$. (non-corrected).

ANALYSIS.

Weight of substance = 0.2103 grams.

$\text{CO}_2 = 0.5598$ grams. $\text{CH}_3 \text{H}_2\text{O} = 0.0881$

Calculated for $\text{C}_6\text{H}_5\text{CH}_2\text{CO}$  = 73.49% H = 4.76%

Found C = 72.46% H = 4.66%

REACTION TO IODINE.

Mixed crystals from alcohol, slowly at N/100,
at once at N/10.

CINNAMOYL DERIVATIVE.

In the preparation of the acid chlorides it has been found convenient to use thionyl chloride in place of PCl_5 , the method used being that given by Hans Meyer, Konstitutions-ermittlung 1916 edition, p.554. Technical thionylchloride obtained from British Drug Houses was purified by distillation over sulphur and anhydrous aluminium chloride. The acid was then mixed with a large excess of thionyl chloride and placed in a tube which could be sealed if necessary. It was rarely found necessary to heat the tube, the reaction going at ordinary room temperatures. The excess thionylchloride was recovered by distillation and the acid chloride then purified by distillation in vacuo.

Cinnamoyl chloride has a B.P. of 154° under 23 mm. Liebermann B 21, 3372, and melts at 35°C .

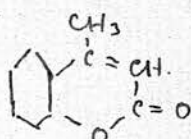
This was then condensed by Einhorn's pyridine method and the derivative separated by pouring into acidified water. It was recrystallised from absolute alcohol in which it is slightly soluble and gives a M.P. of 154°C . (non-corrected).

ANALYSIS./

ANALYSIS.

Wt. taken = 0.2756 grams.

H₂O = 0.1149 grams. CO₂ = 0.7504 grams.

Calculated for C₆H₅CH=CH CO O 

C = 74.50% H = 4.58%

Found C = 74.27% H = 4.63%

REACTION TO IODINE.

Amorphous adsorption slowly from alcoholic solution at N/500. From solutions in alcohol and glacial acetic acid and glacial acetic acid alone, No increase in the sensitivity was observed.

PHENYL ISOCYANATE DERIVATIVE.

Prepared by heating together calculated quantities of phenyl isocyanate and β -methyl umbelliferone. Hans Meyer (1916 edition) p.565. It was recrystallised from ligroin and had a melting point of 155°-156°C.

ANALYSIS.

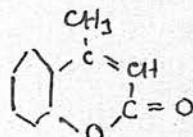
The nitrogen was estimated by the micro-Kjeldahl method.

Weight of substance = 0.02874 grams.

ccs. acid used = 8 ccs.

Blank/

Blank	=	0.9 ccs.
∴ required	=	7.1 ccs.
1 cc. acid	=	0.0002 grams N .
∴ 7.1 ccs.	=	0.00142 grams N .

Calculated for $C_6H_5NHC O O$  N = 4.75%.

Found N = 4.94%

REACTION TO IODINE.

From alcoholic solution bluish black mixed crystals at N/10. At N/100 brownish masses appear slowly after 5 minutes, the remaining solution being brown. If this is observed under the microscope, the particles are seen to be in violent motion and gradually coalesce, forming dark coloured crystals. On further standing it crystallises out without formation of the blue colour.

β -NAPHTHOYL DERIVATIVE.

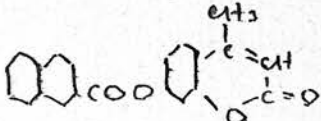
The acid chloride prepared by the thionyl chloride method outlined above was condensed with β -methyl umbelliferone, using Einhorn's method. It was recrystallised from glacial acetic acid, being very little soluble in warm alcohol. M.P. 179°-180°.

ANALYSIS.

Wt. of substance = 0.3678 grams.

H₂O = 0.1363 grams.

CO₂ = 1.0280 grams.

Calculated for  C = 76.37% H = 4.15%

Found C = 76.19% H = 4.12%

REACTION TO IODINE.

From alcohol gives limit of N/500 and from glacial acetic acid N/1000.

ORTHO-CHLOR-BENZOYL DERIVATIVE.

The acid chloride was prepared, condensed with β-methyl umbelliferone and the derivative isolated in the manner outlined for the cinnanoyl derivative. It was recrystallised from alcohol and had a M.P. of 155° - 156°.

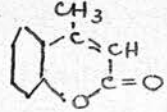
ANALYSIS.

The chlorine was estimated.

Wt. of substance 0.1037 grams.

Wt. of Ag Cl = 0.0473 grams.

Calculated for

C₆H₄Cl CO O  Cl = 11.29%

Found Cl = 11.30%.

REACTION TO IODINE.

With ^a saturated alcoholic solution the limit is N/4,000. On standing the substance gradually separates out in colourless crystals.

META-NITRO-BENZOYL DERIVATIVE.

The acid chloride was prepared, condensed with β -methyl umbelliferone and the derivative isolated in the manner outlined for the cinnamoyl derivative. It was recrystallised from glacial acetic acid. M.P. 210° - 211°.

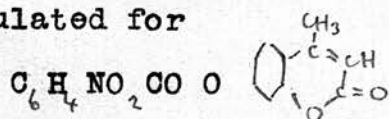
ANALYSIS.

The percentage nitrogen was estimated.

Weight taken = 0.6382 grams.

Volume of nitrogen at 12° and 756 mm. = 5.5 ccs.

Calculated for



N = 4.31%

Found

N = 4.15%

REACTION TO IODINE.

From glacial acetic acid solution at N/5,000 violet. N/1000 blue slowly. N/10,000 crystallises out without adsorption.

PARA-/

PARA-NITRO-BENZOYL DERIVATIVE.

The method of preparation was the same as that used for the preparation of the cinnamoyl derivative. It was recrystallised from glacial acetic acid. M.P. 143°.

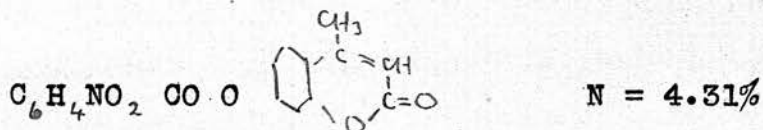
ANALYSIS.

The nitrogen was estimated by the micro-Dumas' method.

Weight taken = 0.0198 grams.

Volume of nitrogen at 150 and 740 mm. = 0.66 ccs.

Calculated for



N = 4.31%

Found N = 4.26%

REACTION TO IODINE.

From glacial acetic acid solution N/1000 at once, N/2000 slowly, N/3,000 violet and at N/5,000 the substance crystallises out without adsorption.

Increased sensitivity is observed in direct proportion to the unsaturation of the group added, the cinnamoyl derivative being more reactive than the phenylacetic derivative.

The/

The effect of the addition of acids on sensitivity has been observed. The more acid grouping of the chlor-benzoyl derivative however does not increase its sensitivity but reduces it in comparison with the benzoyl derivative. It has been found, and this will be dealt with fuller in the next section, that benzoyl umbelliferone has an optimum Ph of 1.2 - 1.4. These facts can only be brought into agreement on the supposition that the adsorption of iodine is dependent on the physical as well as the more purely chemical properties of the substance. The solubility of the chlor-benzoyl derivative in alcohol being only half that of the benzoyl derivative might explain the fact that at a concentration of N/4,500 the benzoyl derivative gives a blue colour while the less soluble chlor-derivative separates out without adsorption. In other words the main difference between these derivatives is that the benzoyl derivative gives a fairly stable colloidal solution whereas the chlor-benzoyl derivative does not.

The inclusion of a basic nitrogen atom as in the phenylisocyanate derivative appears to diminish the sensitivity.

The two nitro-derivatives are extremely insoluble in alcohol and so no adequate comparison with the benzoyl derivative, from the point of view of acidity, could be made. It is however interesting to note that/

that there is a definite difference between these nitro derivatives which cannot be attributed to acidity but to either the position of the nitro-group or to more purely physical causes.

The replacement of the C H group by naphthyl does not increase, but decreases the sensitivity.

EFFECT OF HYDROGEN ION CONCENTRATION
ON SENSITIVITY.

Buffered Ph solutions containing acid phosphate were used. Varying the concentration of the phosphate between wide limits had no effect on either the rate of production or the intensity of the blue colour. Thus any slight difference in the concentration of the phosphate in the solutions used has no effect on the sensitivity. The method used consisted in adding the alcoholic solution of the substance to a solution containing iodine in potassium iodide (the concentration of KI was kept constant) and the Ph solution. If this is done carefully the alcoholic solution floats on the top. The series of test-tubes were then rapidly inverted and in this way it was possible to have under observation five or six solutions of different Ph at one and the same time. The concentration of iodine used corresponded to the limit previously determined, in aqueous solution.

SAPONARIN.

Conditions:- 2 ccs. saponarin solution .08%, 1 cc
N/100 I_2 , 5 ccs. buffer solution.

In this case only two solutions can be observed at once the method being, to pour the contents of two test tubes into test tubes containing saponarin solution.

Results:- Ph 10 - Ph 8, no result; Ph 6 reaches a maximum quickest but rapidly declines to grey blue and finally yellow green; Ph 5 reaches a maximum in slightly longer time and persists longer; Ph 4, green blue which persists and Ph 1.2 - 1.4 gives green. Optimum Ph 4 - Ph 5.

BENZOYL DERIVATIVE METHYL UMBELLIFERONE.

Conditions:- 2 ccs. 0.14% alcoholic solution, 2 ccs
N/1000 I_2 and 5 ccs. buffer solution.

Results:- Ph 10 and Ph 8 gave no result. The order of intensity was observed to be

Ph 1.2 - 1.5 > Ph 4 > Ph 5 > Ph 6.

corresponding to an optimum of Ph 1.2 - 1.5.

CHLOR-BENZOYL DERIVATIVE METHYL UMBELLIFERONE.

Conditions:- 2 ccs. 0.14% alcoholic solution, 2 ccs.
N/1000 I_2 and 5 ccs. buffer solution.

Results:-/

Results:- Ph 5 - Ph 6 gave no colour. Ph 4 very slight, the optimum being observed at Ph 1.2 - 1.5.

INDENOFILAVONE.

Conditions:- 0.2 ccs. 0.05% alcoholic solution,
1 cc. N/1000 J_2 and 5 ccs. buffer solution.

Results:- Ph 10, pink; Ph 8 - Ph 4, violet;
Ph 1.2 - 1.5 pure blue. Optimum observed at Ph 1.2 - 1.5.

2 - NAPHTHOFILAVONE.

Conditions:- 0.2 ccs. 0.05% alcoholic solution,
1 cc. N/1000 J_2 and 5 ccs. buffer solution.

Results:- Ph 10 - Ph 8, no result; Ph 6, blue;
Ph 5, 4, 1.2 - 1.5, give blue but coagulate quickly. The optimum in this case is Ph 6.

Conditions:- 0.2 ccs. 0.4% alcoholic solution,
1 cc. N/500 J_2 and 5 ccs. buffer solution.

Results:- Ph 10, no result; Ph 4 - Ph 8, colour produced only on standing; Ph 1.2 - 1.5, blue produced immediately and corresponds to the optimum.

EFFECT OF IONS ON SENSITIVITY.

The results obtained in the above section on the very marked influence of hydrogen ions on adsorption suggested testing the influence of other ions on adsorptive power. A difficulty here presents itself. Owing to the activity of iodine it is impossible to find a solvent which can be used and yet which is not acted upon by the iodine to produce hydrogen iodide. In the following experiments a fresh dilute alcoholic solution (N/1000) of iodine was used. As this solution was used throughout, as rapidly as possible, the error due to the presence of hydrogen iodide is the same in each case. The method adopted consisted in comparing the rapidity of formation of the blue colour and its intensity in presence of different potassium salts, first with "iodide" as a standard and afterwards with each other. The comparison is most conveniently carried out by inverting simultaneously the respective solutions into a known fixed volume of an alcoholic solution of the substance under examination. In this way using 0.2 Molar solutions the following order was arrived at.

CNS < Cl, Br, NO₃, SO₄, acetate, tartrate, citrate
 < iodide.

CNS entirely inhibits the reaction, iodide is the most/

most active and although slight differences can be detected between the other anions mentioned, these are not sufficiently large to admit of any one being considered more active than another.

Different cations also produce a marked difference in the production of the blue colour. The order being with mono-valent metals $\text{NH}_4 < \text{Na} < \text{K}$.

Since in the case of the substance examined, benzoyl β -methyl umbelliferone, the colour disappears on allowing the solution to stand, it is possible to compare the relative amounts of iodine in the solution before and after adsorption by comparing the intensity of colour obtained by adding chloroform. When this is done it is found that in the case of CNS, iodine molecules as such have disappeared whereas in the case of "iodide" a slight diminution only is observed. The inhibitory action of CNS is therefore in this case to be explained purely by the chemical action of the iodine on the KCNS.

Similar results were obtained on substituting benzylidene phthalide for benzoyl β -methyl umbelliferone.

ULTRAMICROSCOPIC/

ULTRAMICROSCOPIC INVESTIGATION.

The influence of hydrogen ion concentration and of salts on the additive power of a substance for iodine are properties due to the colloidal nature of these substances. Some of the more stable of the colloidal solutions have been examined ultra microscopically, using both the Zigmondy and the cardioid cells but without obtaining very much light on the subject. Most of these colloids are prepared by addition of an alcoholic solution either to water or iodine solution, this gives rise to a large number of micro bubbles and very often before these have been got rid of the substance begins to coagulate. This also made it impossible to observe directly the addition of iodine to the substance and the formation of the blue colour. It was however found possible to observe the transition of the blue colour to water-white in the case of saponarin. To 4 ccs. 0.08% aqueous solution of saponarin 0.4 ccs. N/50 I_2 were added, a blue colour results which disappears after 10 minutes. No obvious change in the size of these particles as indicated by the Brownian movement could be detected.

Of the other substances examined Benzylidene phthalide, /

phthalide, β naphthoic acid derivative, ortho-chlor derivative, meta- and para- nitrobenzoyl derivatives and phenylisocyanate derivative all are negative colloids and the addition of iodine seems to increase the negative charge.

EFFECT OF IODINE CONCENTRATION ON
IODINE ADSORPTION.

Although it is not possible, owing to the minuteness of the iodine taken up, to estimate the amount of iodine in every case, the estimation has been attempted with acetyl-coumarin, coumarin and benzoyl β methyl umbelliferone. The method used was that devised by Barger and Field (loc cit p. 1405) in their investigations on cholalic acid.

TABLE./

BENZOYL DERIVATIVE OF HYDROXY-4-METHYL COUMARIN.

Concentration of glacial acetic acid solution $m = 0.2$ milli mols.

Gram atoms of iodine per litre	C	0.167	0.100	0.0412	0.0329	0.0247	0.0172	0.0086
Gram atoms of iodine per molecule of substance	x/m	0.86	0.845	0.775	9.73	0.55	0.163	0.091

ACETYL COUMARIN.

Concentration of alcoholic solution $m = 0.1$ milli mols.

Gram atoms of iodine per litre	C	0.168	0.133	0.0737	0.0553	0.0424	0.0339	0.0170	0.0086	0.0067
Gram atoms of iodine per molecule of substance	x/m	0.71	0.69	0.642	0.515	0.421	0.369	0.369	0.337	0.326

COUMARIN. (1ST CURVE)Concentration of alcoholic solution $m = 0.3$ milli mols

Gram atoms of iodine per litre C	0.164	0.114	0.0658	0.0329	0.0174	0.0139	0.0104	0.0087	0.0070
Gram atoms of iodine per molecule of substance x/m	0.57	0.57	0.57	0.55	0.417	0.357	0.292	0.258	0.208

COUMARIN. (2ND CURVE)Concentration of alcoholic solution $m = 0.4$ milli mols.

Gram atoms of iodine per litre C	0.164	0.148	0.0823	0.049	0.033	0.0170	0.0132	0.0103	
Gram atoms of iodine per molecule of substance x/m	0.67	0.67	0.63	0.63	0.55	0.4	0.34	0.27	

Up till 1922, when Euler and Myrback A 428, 1 - 24, published their interesting paper on starch-iodide, results obtained by the estimation of the iodine content in solution were of the type found above with benzoyl 4-methyl umbelliferone and with coumarin. These investigators however obtained a stair-case adsorption curve like that obtained with acetyl coumarin. From the above curves it is impossible to state whether or not iodine combines with these substances in definite proportions. It is however clear that if chemical compounds are formed they are only stable in an iodine solution of fixed concentration. This makes their isolation and purification difficult and renders it necessary to adopt indirect methods of analysis which do not admit of great accuracy. The error however will be only in the actual numerical figure arrived at, and also being the same in all cases will not effect the general nature of any particular curve. With acetyl coumarin there appear to be two compounds, one having an iodine content exactly half the other, as is shown by the two upright portions of the curve. With the coumarin curves an experiment was made to see in how far the adsorption was altered by altering the concentration, the ratio of iodine solution to alcohol being kept constant.

Curve/

Curve II representing the largest concentration shows a distinct increase in adsorption. The concentration of potassium iodide has recently been shown to alter the adsorption. Firth and Watson, J. Soc. Chem. Ind. (1923) T 42, 308-310. Thus although in each case the blue colour may be obtained which appears to be homogeneous, the actual iodine content may vary between wide limits.

When one takes into account the unsaturated nature of the substances involved, the marked effect of the spatial arrangements and character of their groups, together with the instability of the addition products under certain conditions and the extreme stability under others; when one realises the colloidal nature of the phenomenon, as exemplified by the hydrogen ion concentration experiments and the effect of different cations and anions, it becomes clear that the solution of the problem must be made on some physico-chemical basis. Some such explanation as the following may be advanced. Owing to the residual valency and the character and spatial arrangement of their groups, certain substances possess a strong attraction towards iodine so that the iodine is fixed probably in its non-associated atomic state (Iodine in this state would probably be blue in colour). The amount fixed depending on the activity of the substance and the conditions under which it was brought into contact with iodine.

