It is therefore obvious that this sticky acid of a resinous nature, which forms a most conspicuous part of sumbul extract and gives the latter its disagreeable properties is not a homogenous body. The presence of vanillic acid in the solution after prolonged standing of the alcohol solution suggested that this phenolic acid represents a part of the structure of the complex resin and a methoxyl determination of the resin acid further indicates this.

Subst. 0.1918 g.: CH_3I = 0.0641. Found: OCH_3 = 4.4%.

Vanillic acid was prepared from this resin acid as follows: thirteen grams were boiled for several hours with 5% alcoholic sulfuric acid. The alcohol was removed by steam distillation. The steam distillate contained a small amount of a blue oil resembling the essential oil.

The contents of the flask were shaken with ether, which was then extracted with solutions of ammonium carbonate, potassium carbonate, and potassium hydroxide. From the first of these extracts vanillic acid melting at 205–206° was isolated. The weight was 0.5 g. The potassium carbonate extract yielded umbelliferon (0.05 g.) melting at 225–227°. Nothing definite could be isolated from the potassium hydroxide solution.

The chloroform extract of the resin (46 g.) was divided into two parts. The first (26 g.) was redissolved in chloroform and extracted with the various alkaline solutions but nothing definite could be separated by this process. Most of the material was extracted with potassium hydroxide solution. Twenty grams were hydrolyzed with 5% alcoholic sulfuric acid. Umbelliferon (0.85 g.) melting at 226–227° was isolated. This was present in glucosidic combination, as 1.5 g. of *d*-phenyl glucosazone melting at 206–207° was isolated.

The ethyl acetate extract of the resin (19 g.) was a dark-colored resinous product. Upon hydrolysis a small amount of umbelliferon melting at 226–227° was isolated, but sugar was not formed. The material is therefore not glucosidic.

The alcohol extract of the resin (18 g.) agreed in its properties with the brown glucosidic powder isolated from the amyl alcohol extract of the constituents soluble in water. Umbelliferon and a reducing sugar were found as products of an acid hydrolysis.

KALAMAZOO, MICH.

[CONTRIBUTION FROM THE LABORATORY OF THE DODGE AND OLCOTT CO.]

SOME DERIVATIVES OF COUMARIN.[1]

BY FRANCIS D. DODGE.

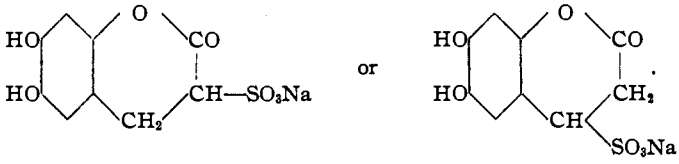
Received November 22, 1915.

The behavior of unsaturated lactones, or more particularly, the coumarins, with the alkaline bisulfites, appears so far to have been studied in but one instance.

[1] Presented at the meeting at New Orleans, Mar., 1915.

Rochleder,[1] in 1863, described a compound derived from aesculetin, to which he gave the formula $C_9H_6O_4.NaHSO_3.{}^1/_2H_2O$. From this a beautifully fluorescent substance termed "aescorcein" was obtained, which was further studied by Liebermann.[2]

The latter showed that the compound of Rochleder's was not an ordinary addition product, like the aldehyde compounds, but was in reality a sulfonic derivative of hydro-aesculetin.



The free sulfonic acid was obtained in crystalline form, easily soluble in water and alcohol, having the composition $C_9H_8O_7S + H_2O$. At 95°, it decomposes smoothly into aesculetin and sulfurous anhydride. By treatment of the sodium sulfonate with acetic anhydride and sodium acetate, a similar reaction occurred, yielding diacetyl aesculetin. Liebermann further states that no analogous derivatives could be obtained from umbelliferon, daphnetin, diethyl aesculetin, or tri-ethyl aesculetinic acid.

The writer has observed, however, that coumarin and limettin (a dimethoxy-coumarin) readily yield well defined and beautifully crystalline sulfonic compounds, which, in several reactions, exhibit a rather interesting behavior.

Sodium Hydrocoumarinsulfonate.—If coumarin is warmed with six parts of a 20% sodium bisulfite solution, on the water bath, with frequent shaking, for fifteen minutes to one-half hour, it dissolves completely, and, on cooling, the liquid solidifies to a mass of crystals, which, after filtering and washing with cold water, are nearly pure. Recrystallization from water effects a further purification, but is accompanied with much loss, owing to the solubility of the compound. From the mother-liquors a further yield is obtained by "salting out" with common salt, and recrystallization.

Calc. for $C_9H_6O_2.NaHSO_3.H_2O$: H_2O, 6.72%; Na_2SO_4, 26.45%. Found: H_2O at 100°, 6.43; Na_2SO_4 (ignition H_2SO_4), 26.14.

Titrations: (1) 1.25 g. required 9.3 cc. 0.5 *N* KOH (calc. 9.15 cc.); (2) 2.1015 g., 15.3 cc. (calc. 15.38 cc.); (3) 10.00 g., 73.6 cc. (calc. 73.2 cc.).

As already stated, the compound is quite soluble in water, a saturated solution at 15° containing about 20% of hydrated salt.

The reaction of the aqueous solution is neutral, but if alkali be added, an immediate reaction occurs, and, as shown above, the compound may

[1] *Wien. Akad. Ber.*, **55**, 819; *J. prakt. Chem.*, **101**, 415.

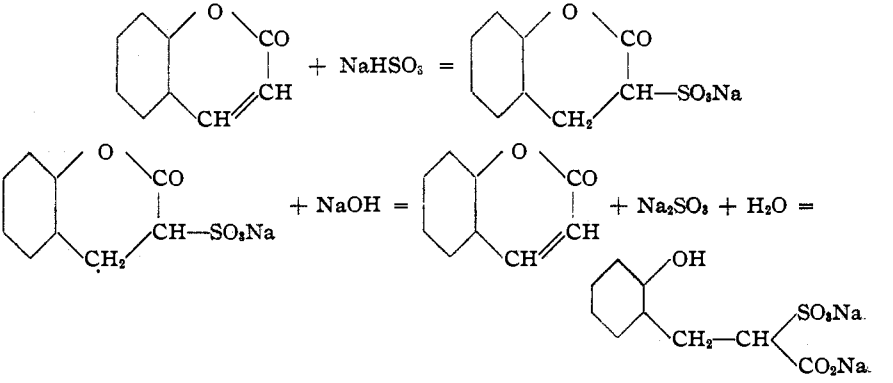
[2] *Ber.*, **13**, 1595 (1880); **14**, 477 (1881); **34**, 2608 (1901); **35**, 2919 (1902).

be titrated with accuracy, giving results which indicate the absorption of one molecule of alkaline hydroxide.

With a concentrated solution of the compound, the addition of a small amount of 0.5 N hydroxide causes an immediate turbidity, which soon becomes a crystalline precipitate of coumarin. On continued agitation, the crystals redissolve and the solution becomes neutral, and further addition of alkali causes a repetition of the phenomena. If the alkali is added quickly, in larger amount, a crystalline magma results which slowly dissolves. Finally, coumarin ceases to separate, and the liquid becomes permanently neutral.

This solution does not contain the original compound, for no precipitate is obtained by saturating it with salt; nor has the sulfonic group been split off, since the iodine absorption is negligible. If, however, the coumarin which is formed by the quick addition of alkali is immediately filtered off, the filtrate shows the presence of much sulfite.

These phenomena have a plausible explanation if we assume the following reactions:



The crystalline sulfonate is, then, a derivative of hydrocoumarin, containing the lactone ring intact. Alkali splits off the sulfo group, liberating coumarin, which then recombines with the neutral sulfite, forming the open-chain hydrocoumaric acid derivative. The position of the sulfo group, whether α or β, remains to be determined. Coumarin, hence, should be soluble in neutral sulfite solution, which, in fact, is the case.

10 g. coumarin and 100 cc. of 25% sodium sulfite solution were allowed to stand at the ordinary temperature, with occasional shaking. After three days, the crystals were almost entirely dissolved, and the still neutral solution behaved in all respects like that obtained by titrating the hydrosulfonate. At 100°, the reaction is quicker, requiring about one-half hour.

Potassium Hydrocoumarinsulfonate.—This salt is readily prepared by warming coumarin with seven parts of 20% potassium bisulfite solution. On cooling, a mass of white needles is formed, which may be recrystallized from hot water. It is less soluble than the sodium salt, the saturated solution at 15° containing 7.6%.

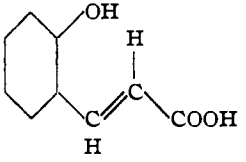
Calc. for $C_9H_6O_2.KHSO_3.H_2O$: H_2O, 6.33%. Found: 6.29.
2.0035 g. required 14.2 cc. 0.5 *N* KOH (calc. 14.1 cc.).

In other properties, it resembles the sodium salt.

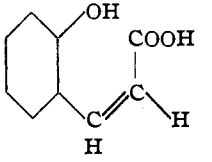
Other Salts.—The barium salt separates as a crystalline precipitate when a solution of barium acetate is added to a strong solution of the sodium salt. It is soluble in about 44 parts of water at 20°.

The copper salt is obtained in pale blue prisms, by slow concentration of the mixed solutions of sodium salt and copper sulfate.

Hydrocoumaric Acid Derivatives.—As is well known, coumaric acid, or *o*-hydroxycinnamic acid is supposed to exist in two stereoisomeric configurations.



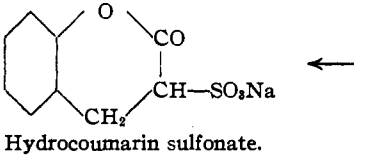
1. Coumaric acid.



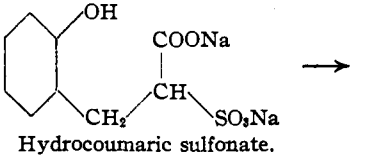
2. Coumarinic acid.

The first, and only stable form, does not readily yield the lactone, coumarin, except indirectly, as, for example, by distillation of the acetyl derivative. In the second case, the proximity of the hydroxyl groups so favors the lactone formation, that the acid itself cannot be isolated; it is assumed to be present in the compounds obtained from coumarin by the action of alkalies at low temperatures.

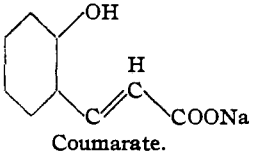
Now, in the case of the hydrocoumaric sulfonate, with the disappearance of the double linkage, vanishes also the possibility of this kind of stereoisomerism. The hydrocoumaric derivatives are, in a sense, intermediate between the coumaric acids, and, by suitable reactions, it should be possible to pass at will to either the coumaric or coumarinic series.



Hydrocoumarin sulfonate.



Hydrocoumaric sulfonate.



Coumarate.

This, in fact, is the case; by splitting off the sulfo group, coumaric acid results, while, by a simple dehydration, the lactone ring is re-established, giving the original hydrocoumarin sulfonate as shown on preceding page.

The solution of sodium hydro-coumaric sulfonate shows little tendency to crystallize. When highly concentrated, a viscid liquid is obtained, which in the desiccator solidifies to an indistinctly crystalline mass, which has not yet been purified. The barium salt, prepared by double decomposition, forms white needles, not very soluble in water.

Formation of Coumaric Acid.—2 g. hydrocoumarin sodium sulfonate were titrated with 0.5 *N* KOH, requiring 14.7 cc., or one molecule. A further addition of 14.7 cc. KOH, or one molecule, was then made, and the solution evaporated to small bulk. The residue was dissolved in 10 cc. water, and the solution titrated with 0.5 *N* HCl, of which 14.6 cc. were required. One molecule of alkali, hence, does not remove the sulfo group, under these conditions. The solution was again concentrated to 10 cc., 29.4 cc. 0.5 *N* KOH (2 mol.) added, and again evaporated, the solution now showing the yellowish green tint characteristic of the coumarates. On titration, 19 cc. 0.5 *N* HCl were required, showing a conversion of about 70%. On addition of concentrated hydrochloric acid to the filtered solution, the coumaric acid was precipitated nearly white, and was identified by the melting point and other properties of that acid. With larger excess of alkali, the yield appears to be almost quantitative.

This smooth reaction offers a convenient method for the preparation of coumaric acid, as the usual process, with sodium ethylate or very strong hydroxide, is somewhat troublesome. For example:

10 g. coumarin are heated with 100 cc. of 25% sodium sulfite until completely dissolved, and the liquid evaporated to about 50 g. 40 g. of 50% potassium hydroxide solution are now added, and the mixture evaporated to dryness on the water bath. The yellowish green residue is then dissolved in water, and the cooled solution acidified with strong hydrochloric acid. The precipitated coumaric acid is filtered and washed with cold water, and after drying, can be conveniently recrystallized from acetone. The yield is about 10 g.

Formation of Coumarin.—The hydrocoumaric sulfonate solution from 2 g. coumarin was evaporated to dryness, pulverized, and mixed with 5 cc. of acetic anhydride. On standing, the mixture becomes thick, and finally solidifies. After two days, the mass was broken up, filtered, and the adhering anhydride allowed to evaporate. The product was soluble in water, but, on adding alcohol, was precipitated in crystals. By recrystallization from water, hydrocoumarin sulfonate was obtained in the usual form.

Or, starting with coumaric acid, the reaction can be carried out in a similar manner.

2 g. coumaric acid were heated with 10 cc. of 30% sodium bisulfite several hours on the water bath, until a cooled sample gave no precipitate of acid on the addition of hydrochloric acid. The liquid was then evaporated to dryness, and the product pulverized. On treatment with acetic anhydride, the same behavior was observed, as with the product from coumarin. The yield of hydrocoumarin sulfonate, crystallized from water, was 1.75 g.

We have, then, here a cycle of reactions by which to pass from coumarin to coumaric acid, or *vice versa*.

Coumarin $\rightleftarrows$ Hydrosulfonate $\rightleftarrows$

Hydrocoumaric sulfonate $\rightleftarrows$ Coumaric acid.

The quantitative recovery of coumarin from the hydrosulfonate presents some difficulty, owing to the rapidity with which it is attacked by the neutral sulfite. Working quickly with ice-cold solutions, the yield is only about 30% of the theoretical; decomposition with barium hydroxide gave a little better result, the yield reaching 40%.

The hydrocoumarin sulfonate is also decomposed by alkaline carbonates, at ordinary temperatures—an exceptional reaction for sulfonic derivatives. On adding strong sodium carbonate solution to a 20% solution of the sodium salt, the mixture remains for a short time clear, then becomes turbid, and a little later the liquid is filled with a net-work of fine coumarin crystals. After a few hours, however, the crystals have entirely disappeared.

The regeneration of coumarin from the hydrosulfonate appears to be quantitative when the latter is heated at 130–160°, the following reaction taking place:

$$2C_9H_7O_2NaSO_3 = 2C_9H_6O_2 + Na_2SO_3 + SO_2 + H_2O$$

The evolution of sulfurous anhydride begins at about 120°, and the reaction is complete after two hours' heating at 150–160°. From the residual mixture of coumarin and sodium sulfite, the former is easily isolated by extraction with ether.

Limettin Derivative.—Limettin, also known as "citraptene," is a dimethoxycoumarin, $C_{11}H_{10}O_4$, easily obtained from the sediment which is deposited from the expressed oil of limes. By crystallization from acetone, it is deposited in yellow needles, melting at 148° (uncorr.). It can also be purified as follows:

10 g. crude limettin are shaken with 100 g. 5% sodium hydroxide solution, until no more dissolves. This may require some days. After filtering from dark resinous matter, 12 g. sodium bicarbonate are added. On standing, limettin gradually separates in yellowish white crystals, the yield from the crude being about 75%. Repetition of the process gives a still lighter colored product. A pure white preparation may be obtained from the hydrosulfonate, as described below.

Hydrolimettin Sodium Sulfonate.—With bisulfite solution, limettin does not react as readily as coumarin. At 100°, solution takes place very slowly, and a higher temperature was found more convenient.

10 g. limettin and 80 g. 20% sodium bisulfite solution were boiled gently one-half hour, till all was dissolved, except a little resin. The liquid was filtered while warm, and allowed to crystallize, finally at a low temperature. The resulting yellow crystals (7.5 g.), were recrystallized from two parts hot water. The sulfonate is thus obtained in fine, large plates, having the composition $C_{11}H_{10}O_4.NaHSO_3.4H_2O$.

(1) 4.8365 g. air dry, lost at 100°, 18.65% (calc. 18.84%).
(2) 1.5355 g. dried, required 9.7 cc. 0.5 *N* KOH (calc. 9.9 cc.).

The salt is soluble in 6 parts water at 20°, and is precipitated on addition of common salt. On titrating the solution with 0.5 *N* alkali, limettin is immediately thrown out, and redissolves much more slowly than is the case with coumarin, owing no doubt to the very slight solubility of limettin in water. Attempts to prepare the limettic acid, corresponding to coumaric acid, were unsuccessful. An acid product was obtained, but it could not be crystallized. Limettin dissolves slowly in a 25% solution of sodium sulfite at 100°; the solution is not precipitated by salt, nor does it yield crystals when concentrated.

Hydrolysis of Coumarin.

The phenomena observed when one attempts to hydrolyze coumarin quantitatively in the usual manner with alkaline hydroxides, are peculiar and of some interest. In fact, the uncertain results frequently encountered in the titration of lactones have already been noted by Baeyer,[1] and are not surprising, when one considers that the tendency to lactone formation is opposed to the hydrolysis, and a condition of equilibrium between lactone and alkaline salt is to be expected.

If coumarin, in alcoholic solution, is titrated at the ordinary temperature with 0.5 *N* alkali, the neutralization is found to be very slow. For example: 2.053 g. coumarin were dissolved in 10 cc. alcohol, and 0.5 *N* alcoholic potassium hydroxide added slowly at intervals, keeping the solution just alkaline to phenolphthalein. After 24 hours, 1.6 cc. had been used, indicating 5.6% coumarin converted into salt.

With excess of alkali, the conditions are different; the hydrolysis appears to be complete; and the titration can be made with approximate accuracy. But as soon as the excess of alkali is neutralized, formation of coumarin begins and continues until the point of equilibrium is reached, the solution becoming alkaline. If, now, the free alkali be again neutralized, a further production of coumarin is observed, and so on until a permanent equilibrium between the coumarin and the salts present is attained, which point depends, of course, on the concentration, temperature, etc.

[1] *Ber.*, 30, 1956 (1897).

If, at this point, the free coumarin be removed, as for example, by extraction with ether, the equilibrium is again disturbed, and a further liberation of coumarin occurs.

A remarkable feature of the reaction is the slowness with which it progresses, and it would seem that a careful study, by physico-chemical methods, would not be without interest.

In the examples given below, it will be noticed that the results of the first titration are always high. The same behavior was noticed in the titration of coumaric acid, and it is possible that the hydroxyl group, having phenolic functions, may, in this case, influence the reaction, though it is well known that salicylic acid, also an orthohydroxy acid, gives very exact titration values. But the aromatic oxy-acids show a varying behavior in this respect, as already noted by Traube,[1] and a further discussion of this point is reserved for a subsequent communication.

Titrations:

1. Coumarin, 1.05 g. Heated one hour at 100° with 26.3 cc. 0.5 *N* alcoholic KOH, then 25 cc. water added, and immediately titrated.

Time	0	10 min.	1 hr.	2 hr.	18 hr.	24 hr.	114 hr.	162 hr.
Total 0.5 *N* HCl	10.9 cc.	12.0 cc.	14.0 cc.	15.5 cc.	18.2 cc.	20.9 cc.	24.0 cc.	24.2 cc.
Coumarinic acid %	107.0	99.4	85.5	75.1	56.3	37.5	15.9	14.9

The alcohol was now evaporated, the solution chilled, the coumarin filtered off, and washed. On standing, the solution became again alkaline, and after 5 days appeared to have reached equilibrium, showing a total of 25.15 cc. 0.5 *N* acid, or 8% coumarinic acid. The solution was now extracted with ether, after which it became slightly alkaline.

Finally, neutrality was reached with 25.25 cc. 0.5 *N* acid, or 7.3% of coumarinic acid. In the solution, evaporated till free from coumarin, no coumaric acid could be detected.

2. Coumarin, 2.019 g., 50 cc 0.5 *N* alc. KOH, heated 2 hours. Titrated without addition of water, and the solution heated at 90–100°.

Here, immediate titration showed 101.9% acid, with equilibrium after 48 hours, at 19.8% acid.

3. Coumarin, 2.077 g., 25 cc. 0.5 *N* alc. KOH, heated 2 hours. Here, although the amount of alkali was insufficient for complete hydrolysis, the solution was still alkaline, and indicated 61% coumarinic acid.

4. Limettin, 2.073 g., 40 cc. 0.5 *N* alc. KOH, heated 2 hours.

Time	0	4 hrs.	20 hrs.	44 hrs.	60 days
Total 0.5 *N* HCl	19.4 cc.	23.7	28.7	31.9	34.2
Limettinic acid %	102.3	80.9	56.1	40.2	28.8

At the end of 4 hrs. limettin commenced to separate in fine needles.

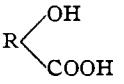
These titrations, of course, give merely a "sketch" of the hydrolysis, no attempt having been made to secure uniformity of conditions.

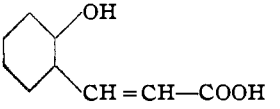
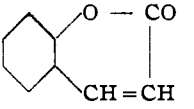
[1] *Ber.*, 31, 1566 (1898).

An analogous reaction takes place when sodium bicarbonate is added to an alkaline solution of coumarin. For example, 5 g. of coumarin were dissolved in 60 cc. of warm 5% sodium hydroxide. When cold, 5 g. sodium bicarbonate were added, and the solution let stand. Coumarin soon began to crystallize out, and after five days the yield amounted to 3.46 g., or about 70%.

On the Lactone Formation.—The lactones may be considered as esters, in which the ester formation is intramolecular.

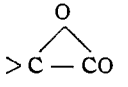
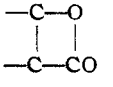
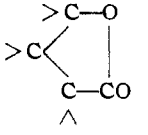
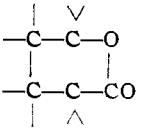
thus: Ester: $R{-}OH + R'COOH = R{-}O{-}CO{-}R' + H_2O$
(alcohol) (acid) (ester)

Lactone:  = + H_2O
(oxy-acid) (lactone)

Coumarin:  =  + H_2O
(Coumarinic acid) (Coumarin)

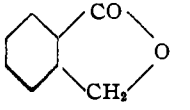
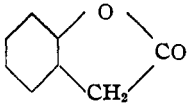
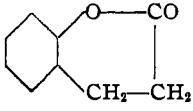
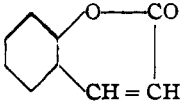
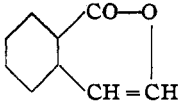
The velocity of the typical ester formation depends not only on the concentration and the temperature, but also on the chemical nature of the constituents. Menschutkin has shown that the velocity is greatest with primary alcohols, and least with the tertiary alcohols. With phenols, the reaction practically does not take place. The structure of the acid is also of importance, as was shown by Victor Meyer, in his studies of the esterification of the ortho-substituted acids.

With the lactones, the spacial relations appear to have a preponderating influence. Thus, among the simpler fatty derivatives, the following rules seem to hold:

1. From α oxy-acids, α lactones.....  (not known)
2. From β oxy-acids, β lactones .....  (a few cases)
3. From γ oxy-acids, γ lactones.....  (lactone stable) acid unstable
4. From δ oxy-acids, δ lactones.....  (lactone unstable) acid stable

Thus, in accordance with the "Spannungs-Theorie" of Baeyer, according to which the simple five atom ring is the most stable, the lactones in the fatty series are also the most readily formed and exhibit the greatest stability.

In the aromatic series, where the benzene and the lactone rings have two atoms in common, somewhat different conditions prevail. For example:

	γ lactone (phthalide)	Not formed spontaneously. Acid stable to 100°.
	γ lactone (keto-hydro-coumarone)	Not formed spontaneously. Acid stable to 137°.
	δ lactone (hydro-coumarin)	Not formed spontaneously. Acid stable to 100°(?).
	δ lactone (coumarin)	Formed spontaneously. Acid unknown.
	δ lactone (iso-coumarin)	Unstable. Acid unknown.

It is evident that a five-ring, of which two atoms also form part of a benzene ring, is under an internal tension rather different from that existing in a simple ring. It is also apparent, from the dissimilarity of coumarin and iso-coumarin, that some unknown factor renders the configuration of the former an especially favorable one.

Coumarinic acid has the combined functions of a phenol and a cinnamic acid, and we may consider the coumarin formation as analogous to the ester reaction between phenol and cinnamic acid. But the favorable spacial relations cause the former reaction to occur spontaneously, even in alkaline solution, while in the latter case, no reaction takes place even in the presence of acid catalyzers.

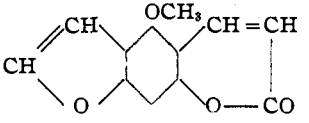
Recognition of Coumarins.

Although the coumarin formation in alkaline solution cannot be considered as strictly characteristic, inasmuch as other lactones are known to exhibit the same behavior, though to a much less degree, it is yet allowable to assume that, in cases where this peculiar reaction is observed, some member of the coumarin series is probably present.

For example, it can hardly have escaped the attention of anyone who

has had occasion too make the usual ester determination on the oil of bergamot, that the end reaction, on titration after saponification, is not sharp. If the solution be set aside, it will be found, after a short time, again alkaline, and will require 0.2 to 0.3 cc. 0.5 N acid for neutralization. On standing, the alkaline reaction reappears, exactly as described for coumarin. To reach permanent neutrality may require 48 hours or more, and the difference between the first and final values may amount to 1 or 2%, if calculated as linalyl acetate.

Other oils containing esters, such as lavender, peppermint, birch, pine-needle, etc., which have been *distilled*, do not show this behavior. Bergamot, however, is an *expressed* oil, and contains 5–6% of nonvolatile matter. In the latter must be found the cause of the phenomenon, and, in fact, the crystalline "bergaptene," $C_{12}H_8O_4$, isolated from bergamot residues by Mulder,[1] and studied by Pomeranz,[2] is formulated by the latter as a methoxy-coumaron-coumarin, derived from phloroglucin, and having possibly the structure



(position of side chains not determined).

In addition to coumarin, bergaptene, limettin, umbelliferon, daphnetin, aesculetin, "chrysatropic acid," already identified as natural products, it appears very probable that other coumarins are yet awaiting recognition. Possibly an example may be found in the "xanthoxylin" of H. M. Gordin.[3]

Gordin isolated from the bark of the northern prickly ash (*Xanthoxylum fraxineum*), a crystalline compound, $C_{15}H_{14}O_4$, with melting point at 132°. He showed the presence of one methoxyl group, but could not determine the nature of the other three oxygen atoms, deciding rather against a lactone formula. Yet he found xanthoxylin to exhibit exactly the behavior on hydrolysis which I have described for coumarin. On treatment with excess of alcoholic alkali, it takes up slightly more than would be required for a monobasic acid. On standing the alkali is slowly liberated, and may be titrated, etc., until the entire amount of xanthoxylin has been set free.

In spite of Gordin's reasons for rejecting the lactone formula, the writer is inclined to believe that xanthoxylin will be found to be a coumarin, and, indeed, possibly a homolog of bergaptene, as a comparison of the formulae will show.

[1] *Ann.* 31, 70 (1839).
[2] *Monatsh.*, 12, 380 (1891).
[3] THIS JOURNAL, 28, 1649 (1906).

Finally, it may not be superfluous to recall the fact that many substances, of great interest by reason of their physiological properties, have been found to possess a lactone structure. Cantharidin, anemonin, aesculin, digitoxin, helenin, santonin, picrotoxin, strophanthin, sedanolid, artemisin, are examples, and it seems not unlikely that there may be some intimate causal relation between the mobility of the lactone ring and the observed physiological activity.

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SPECTROPHOTOMETRIC STUDY OF COPPER COMPLEXES AND THE BIURET REACTION.

BY PHILIP ADOLPH KOBER AND ARTHUR B. HAW.

Received October 1, 1915.

CONTENTS.—I. Introduction. II. Absorption in the ultraviolet: (*a*) Technic; (*b*) Results. III. Absorption in the visible spectrum: (*a*) Technic; (*b*) Results. IV. General discussion. V. Summary.

I. Introduction.

In the first paper[1] on the configurations of the copper complexes and their relation to the biuret reaction, it was found that the copper complexes of amino derivatives and other similar substances could be divided, according to their color, into three classes. (1) Blue, (2) purple—called "semi-biuret"— and (3) red—called "biuret." The first class was also characterized by the fact that there were in each complex *2 nitrogen groups so placed*, that by forming "stable" rings,[2] *they could combine with the copper*. The complexes of the *second class had 3 such groups* and the *third class had 4*.

All told, 114 substances were found which fitted in this classification, of which 89 were studied by the authors. In short, all of the data found pointed to but one explanation, *i. e.*, that the copper in the red complexes was the central atom and that 4 nitrogen groups were combined with it coördinately in the sense used by A. Werner. The nitrogen groups could be either amino, imino, imide, or amide. As the coördination number of copper is four, the purple complexes probably contain besides 3 nitrogen atoms, one aquo or hydroxy group attached to the copper. The bluish tint of these purple complexes is undoubtedly due to the latter groups.[3] Similarly the blue complexes may have two hydroxy groups attached to the copper.

That the nitrogen plus copper is not a red producing chromophore and that the red colors are produced, simply by removing the blue produc-

[1] P. A. Kober and K. Sugiura, *Am. Chem. J.*, **48**, 383 (1912); also original communications, *8th Intern. Congr. Appl. Chem.*, **6**, 165 (1912).

[2] 4, 5, or 6 membered rings.

[3] See discussion on page 471.