XXVII.—*On Limettin.*

By WILLIAM A. TILDEN, D.Sc., F.R.S.

IN a paper read to the Chemical Society, nearly two years ago (Trans., 1890, **57**, 323), Mr. C. R. Beck and I described, under the name *limettin*, a substance obtained from the fruit of the lime. The original specimen of raw material having been exhausted, I applied to Mr. Joseph Sturge, a director of the Montserrat Company, and he kindly supplied me with a considerable quantity of the deposit filtered from oil of limes after delivery in this country. This material, which presented the colour and aspect of butter, was much cleaner than the dried deposit first operated upon and, though saturated with the essential oil, was more easily purified.

A small portion of it purified by crystallisation, first from alcohol rendered alkaline by the addition of potash, and subsequently from pure methylated spirit, was found to melt at 146—147°. The melting point of the specimen described in the former paper was 121°. Hence, though the analyses given were closely concordant, the question arose whether the substance first examined was a mixture of two closely allied substances, or whether it was merely contaminated with a small quantity of impurity which brought down the melting point. The appearance of both specimens was the same. In the first series of experiments, the limettin, supposed to be pure, was found to be converted by boiling with pretty strong caustic alkali into a substance having the same appearance and general properties, but melting at 147°, and this was supposed to be deacetyllimettin. It now turns out, however, that this is the pure limettin itself.

To test this question, 18·46 grams of the original substance, which after the whole had been fused in the air-bath was found to melt at 123°, was boiled for about 20 minutes with 54 grams of caustic soda and 150 c.c. water. The alkaline yellow solution was then precipitated with hydrochloric acid, and the whole of the precipitate carefully collected, washed, and dried. It weighed 18·29 grams. The mother liquors were found to contain mere traces of acetic acid, together with a small quantity of a sticky, brown matter soluble in ether. The treatment with soda, therefore, does not extract an appreciable amount of anything from limettin, but it renders the latter more easily capable of purification, for after crystallising the recovered substance, once from glacial acetic acid (which retained a little brown matter) and twice from methylated spirit, it melted at 147—148°. After recrystallisation, it melted sharply at 147·5°.

Hence it appears that the original limettin, melting at 121—123°, was contaminated with a small quantity of a substance which is altered by heating with caustic alkali, but is precipitated from the solution by acids, along with the real limettin. This contamination probably consists of a kind of vegetable wax, of which some quantity was separated in the course of the crystallisations, and which seems to be soluble to about the same extent as limettin in all the solvents, alcohol, acetic acid, toluene, &c., which are available.

The waxy matter separated as far as possible from limettin melts below 100°.

The new supply of raw material was treated in the following manner:—It was first steam-distilled as long as oil came over. The residual yellow mass was semifluid, but solidified on cooling. It was then treated with successive small portions of boiling methylated spirit, and the undissolved crude limettin purified by crystallisation from benzene or toluene, and again from alcohol. The yellowish, waxy matter is very difficult to remove, but even when quite pure, limettin is not white. It crystallises in prisms, or tufts of needles, which are of a pale-straw colour. It is easily soluble in hot alcohol, acetic acid, benzene, or toluene, but dissolves very sparingly in water or in light petroleum. Weak solutions of the pure substance in alcohol, acetic acid, toluene, or water exhibit a strong violet fluorescence, the appearance of which is good evidence of its purity, as the fluorescence is masked completely by the presence of a very small quantity of the attendant yellow substance.

Two combustions of the new substance crystallised from alkaline alcohol, and from toluene, gave the following results:—

Exp. No.	Substance analysed.	CO_2 obtained.	H_2O obtained.	Carbon per cent.	Hydrogen per cent.
1....	0·2924	0·6815	0·1339	63·54	5·06
2....	0·2922	0·6814	0·1337	63·58	5·06
	Mean percentages			63·56	5·06

With the view of studying the structure of limettin, among other experiments tried, a cold alkaline solution was treated with successive doses of potassium permanganate. When an excess of the oxidising agent is used, abundance of oxalic acid and a very little acetic acid is formed, and nothing else. With a smaller quantity (25 grams of permanganate in 800 c.c. of water to 50 grams of limettin and 50 grams of potash in 200 c.c. of water), only brown tarry matters were formed, but a quantity of limettin remained unoxidised. This, when crystallised from alcohol, gave at first, and while the solutions were still brown, brilliant leaflets or scales totally different in aspect from pure limettin. But in proportion as the impurity was removed, the substance recovered, its usual form of pale-yellow prisms (m. p. 147°), the solutions of which exhibited the characteristic fluorescence.

The presence of a very small quantity of the brown product of oxidation seemed to cause a complete change of crystalline habit.

After this treatment, the limettin was deemed very pure, and was, therefore, analysed. The following are the results :—

Exp.	Substance.	CO_2.	H_2O.	C p. c.	H p. c.
3	0·3008	0·7029	0·1343	63·73	4·95
4	0·3013	0·7080	0·1359	64·05	5·01
5	0·1328	0·3092	0·0560	63·48	4·67
6	0·2011	0·4671	0·0850	63·35	4·69
		Mean percentages		63·65	4·83

These numbers agree with the results of the first two combustions.

When melted limettin is heated, it boils at about 200°, a considerable quantity distilling unchanged, even under ordinary atmospheric pressure. That it is unchanged is shown by its characteristic fluorescence, and by the melting point remaining unaltered, provided it is carefully recrystallised. In the former paper, the distilled product was supposed to contain about 1 per cent. more carbon, but the following analyses of the carefully purified substance show that this was an error due to imperfect purification :—

Exp.	Substance.	CO_2.	H_2O.	C p. c.	H p. c.
7	0·2268	0·5310	0·1005	63·85	4·92
8	0·2241	0·5224	0·1007	63·57	4·99
9	0·2342	0·5464	0·1049	63·63	4·97
		Mean percentages		63·68	4·98

The mean results of the two series of combustions quoted in the former paper were—

Carbon	63·14 and 63·29
Hydrogen	4·86 and 4·71

The substance analysed was, as already explained, not quite pure. The three series of analyses of the pure compound give the percentages as stated above, namely:—

Exps. ..	1—2.	3—6.	7—0.
Carbon	63·56	63·65	63·68
Hydrogen	5·06	4·83	4·96

The formula adopted on the results of the old analyses was $C_{16}H_{14}O_6$. But there appears now to be no doubt that the composition of limettin is really expressed by the formula $C_{11}H_{10}O_4$, which corresponds equally well with the results of experiment:—

	Calculated for	
	$C_{11}H_{10}O_4$.	$C_{16}H_{14}O_6$.
Carbon	64·07	63·57
Hydrogen	4·85	4·63

Determination of Molecular Weight.

I. Depression of freezing point of acetic acid:—

Limettin taken	0·8579
Acetic acid...........	103·6161

Freezing point of acetic acid..........	3·120°
,, solution............	2·970
Depression	0·150

$$K = 39,\ M = \frac{39 \times 0{\cdot}8579 \times 100}{0{\cdot}15 \times 103{\cdot}61} = 215.$$

II. Depression of freezing point of benzene:—

Limettin taken	0·7815
Benzene ,, ...	85·609

Freezing point of benzene............	1·560°
,, solution............	1·350
	0·210

$$K = 49,\ M = \frac{49 \times 0{\cdot}7815 \times 100}{0{\cdot}21 \times 85{\cdot}609} = 213.$$

The formula $C_{11}H_{10}O_4$ gives the molecular weight 206.

Action of Bromine on Limettin.

When into a cold solution of limettin in benzene a solution of bromine in benzene is allowed to fall, drop by drop, an orange-coloured precipitate is formed so copiously that the liquid often becomes semi-solid. After an excess of bromine has been added, the precipitate shrinks, and becomes paler in colour. Hydrogen bromide is immediately evolved. In order to prepare the bromo-derivative, the solution of limettin in benzene, toluene, or glacial acetic acid is poured into a solution of bromine in the same solvent, keeping the bromine throughout in excess. The precipitate is allowed to remain, in the presence of a slight excess of bromine, for about half an hour, and is then filtered off and crystallised from chloroform. It is very slightly soluble in spirit of wine. The bromo-compound is never quite white; it forms scales or small prisms.

Analysis.

Preparation 1.—Toluene used as solvent:—

0·2337 gram gave 0·2468 gram AgBr = 44·94 per cent. Br.

Preparation 2.—Acetic acid as solvent:—

0·3589 gram gave 0·3706 gram AgBr = 43·95 per cent. Br.

Preparation 3.—Acetic acid as solvent:—

0·3411 gram gave 0·3486 gram AgBr = 43·49 per cent. Br.

A portion of Preparation 2 was dissolved in chloroform, bromine added, and the mixture heated in a sealed tube for about six hours at 120—130°, and the product analysed.

0·3406 gram gave 0·3590 gram AgBr = 44·81 per cent. Br.

Under the most favourable circumstances, therefore, the compound contains not more than 44·8 to 44·9 per cent. of bromine, and this agrees closely with the number calculated from the formula $C_{11}H_8Br_2O_4$, which requires 43·95 per cent. of bromine. This point required careful examination, because it was found that chlorine produces *tri*-chloro-substitution compound at once.

Three other specimens of the bromo-compound were submitted to combustion, and gave the following results:—

	I.	II.	III.	Calculated for
		(With silver coil in front.)		$C_{11}H_8Br_2O_4$.
Carbon	36·85	36·59	36·75	36·23
Hydrogen ..	2·64	2·74	2·91	2·19
Bromine ...	—	—	43·23	43·95

The bromo-compound melts at 257° with decomposition.

Action of Chlorine on Limettin.

Limettin was dissolved in glacial acetic acid and dry chlorine passed into the cold solution. A precipitate was at first formed which redissolved in presence of an excess of chlorine. After standing for some hours, the addition of water threw down a white precipitate, which was washed and crystallised from boiling methylated spirit, in which it was found to be more soluble than the bromo-derivative.

First Preparation.

Exp.	Substance.	AgCl.	Percentage of Cl.	Mean.
1	0·2055	0·2846	34·25	—
2	0·0850	0·1189	34·58	34·41

Second Preparation.

	Substance.	CO_2.	H_2O.	C p. c.	H p. c.
3	0·2533	0·3930	0·0548	42·24	2·40

The formula $C_{11}H_7Cl_3O_4$ requires—

Carbon	42·64
Hydrogen	2·26
Chlorine..............	34·40

The chloro-compound crystallises in perfectly white, silky needles. It melts at 188·5°.

Action of Chlorine on Dibromolimettin.

The dibromo-compound suspended in glacial acetic acid is treated with excess of chlorine in the cold. The bromo-compound dissolves, and on addition of water, after some time, a mass of colourless, crystalline needles is deposited. After recrystallisation from toluene, the new compound melted at 202°.

0·1355 gram substance gave 0·1737 gram of mixed bromide and chloride of silver.

The amount required by the formula $C_{11}H_7Br_2ClO_4$ is 0·1766. Probably, therefore, the replacement of the third atom of hydrogen by the chlorine was not quite complete, but the result is sufficiently near to establish the nature of the compound.

Action of Oxidising Agents on Limettin.

A 5 per cent. solution of chromic acid oxidises limettin completely into carbon dioxide and acetic acid. The action of permanganate has already been described (p. 346).

Nitric acid produces a red, resinous substance, which, by further action of the strong acid, dissolves, and a pale-yellow solution, containing abundance of oxalic acid, results. Dilute nitric acid (1 vol. of acid of sp. gr. 1·4, diluted with eight times its volume of water) slowly attacks limettin when gently heated. Effervescence, due to escape of carbon dioxide, continues for some time, and a yellow precipitate is formed. This precipitate, filtered off and dissolved in boiling methylated spirit, gives tufts of small, yellow prisms, amounting to about 30 per cent. of the limettin employed.

Analysis.

Substance.		CO_2.	H_2O.	C p. c.	H p. c.	N p. c.
0·2917	gave	0·5657	0·1003	52·86	3·80	—
0·2950	„	0·5733	0·0983	52·98	3 70	—
0·3170	„	15 c.c. N_2 at 19°; bar. 750·5 mm.				= 5·4
0·2226	„	11·6 c.c. N_2 at 20°; bar. 749·1 mm.				= 5·9

These results correspond with the formula of a mononitro-limettin, $C_{11}H_9(NO_2)O_4$, which requires

Carbon	52·54
Hydrogen	3·60
Nitrogen	5·53

Action of Caustic Potash on Limettin.

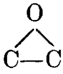
As explained in the former paper (*loc. cit.*, p. 325), limettin fused with potash gives phloroglucol with acetic acid and a little formic acid. No other acid has been detected, although the proportion of alkali and the time and temperature of fusion have been varied.

Action of Hydriodic Acid on Limettin.

Hydrochloric acid, even at 160°, produces no volatile alkyl chloride, but when limettin is boiled with about 10 times its weight of hydriodic acid of sp. gr. 1·96, it evolves methyl iodide readily. 2 grams of limettin boiled with the acid, and the evolved vapour conducted first up an inverted Liebig condenser, which was allowed to become warm, and then into a bulb-tube kept at a temperature below 0°, gave 2·37 grams of the methyl iodide.

On the assumption that one methoxyl group is present, 2 grams of limettin should yield 1·37 grams of methyl iodide. It is, therefore, evident that two such groups are present. This hypothesis requires 2·75 grams of methyl iodide.

Action of Hydrating Agents.

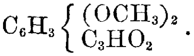
Much diluted hydrochloric or sulphuric acid has no effect on limettin. Potash dissolved in methyl alcohol, heated with limettin at 150°, leaves the greater part of it unchanged. The solution yields very little acetic acid, and sufficient phloroglucol to produce a purple colour upon a slip of deal wood. Moderately strong sulphuric acid gave the best results. 10 grams of limettin were heated to boiling for a few minutes with a mixture of 30 c.c. sulphuric acid and 30 c.c. water. The orange-coloured solution was poured into about 400 c.c. of water, and boiled for some time. The liquid gives a purple colour to deal moistened with hydrochloric acid. On cooling, the orange-coloured, resinous precipitate was filtered off, and the filtrate extracted with ether. The ethereal extract dissolved in a little alcohol mixed with water, and filtered from resin, gave, on standing, a small crop of prismatic crystals. A few more were obtained by dissolving the orange resin in alcohol, and proceeding in the same way. The total yield, however, was very small, probably not more than a gram altogether. As it was necessary to economise, the new substance was not submitted to combustion, but was purified, as far as possible, by recrystallisation, and the product, which was nearly white, was heated at 100° with acetyl chloride in a sealed tube. On opening the tube, there was abundant escape of hydrogen chloride, and on evaporating the excess of the liquid, a residue was obtained which crystallised from alcohol in small, colourless, brilliant prisms. To determine the acetyl in this compound, it was dried over H_2SO_4, and 0·74 gram was boiled with 25 c.c. of 5 per cent. aqueous potash for about 20 minutes. The solution was then carefully distilled with dilute sulphuric acid, the distillate neutralised with pure barium carbonate, filtered, and the solution evaporated to dryness. The residual barium acetate, after drying at 139°, weighed 0·648 gram, and after treatment with sulphuric acid, gave 0·588 gram of barium sulphate. These results correspond respectively to 29·4 per cent. and 29·3 per cent. of acetyl C_2H_3O. The formula $C_{11}H_{10}(C_2H_3O)_2O_5$ requires 29·7 per cent. It is, therefore, the diacetyl derivative of the hydroxy-compound formed from limettin by the addition of the elements of 1 mol. of water. As stated in the former paper, acetyl chloride has no action upon limettin itself, and acetic anhydride with zinc chloride causes blackening. Hence, limettin contains no hydroxyl, but probably includes the group , which, by the action of sulphuric acid and water, is converted into HO·C—C·OH.

Limettin is not altered by phenylhydrazine. When boiled with alcoholic potash and excess of ethyl iodide for about five hours, a sticky, brown resin was formed, but the greater part of the limettin remained unchanged. It, therefore, seems to be established that this compound contains no :CO group in any form corresponding to a ketone or an acid.

Limettin in alcoholic solution is unchanged by the action of sodium amalgam. It gives no coloration with ferric chloride.

Conclusion.

The evidence afforded by the results of the experiments described in this paper leads to the conclusion that limettin does not agree with any known compound. It has been shown to have the molecular formula $C_{11}H_{10}O_4$, which may be dissected in the following manner:—



$$C_6H_3\begin{cases}(OCH_3)_2\\C_3HO_2\end{cases}.$$

It behaves as a saturated compound, and, therefore, the structure of that part of the molecule which is represented above by the symbols C_3HO_2, is not at present clear, but further experiments are begun, with the object of clearing up this point.

In conclusion, I desire to express my obligations to Mr. J. H. Wilson and to Mr. J. H. Millar, students in the laboratories of the Mason College, for the greater part of the analytical work required in this investigation.

Mason College, Birmingham,
February 12, 1892.