of acids when boiled, in dilute alcohol solution, with the alkali salts of the acids. Many of these esters are readily purified by recrystallization from more or less dilute alcohol and have convenient melting points.

The following esters have been thus prepared:

p-Nitrobenzyl formate, $HCO_2.CH_2C_6H_4NO_2$, m. 31°;
Acetate, $CH_3CO_2.CH_2C_6H_4NO_2$, m. 78°;
Propionate, $C_2H_5CO_2.CH_2C_6H_4NO_2$, m. 31°;
Butyrate, $C_3H_7CO_2.CH_2C_6H_4NO_2$, m. 35°;
Benzoate, $C_6H_5CO_2.CH_2C_6H_4NO_2$, m. 89°;
o-Toluate, $o\text{-}CH_3C_6H_4CO_2.CH_2C_6H_4NO_2$, m. 90.7°;
o-Nitrobenzoate, $o\text{-}NO_2C_6H_4CO_2.\text{-}CH_2C_6H_4NO_2$, m. 111.8°;
o-Chlorobenzoate, $o\text{-}ClC_6H_4CO_2.CH_2C_6H_4NO_2$, m. 106°;
Anthranilate, $o\text{-}NH_2C_6H_4CO_2.CH_2C_6H_4NO_2$, m. 205°;
p-Bromobenzoate, $p\text{-}BrC_6H_4CO_2.CH_2C_6H_4NO_2$, m. 139.5°;
2,4-Dinitrobenzoate, $2,4\text{-}(NO_2)_2C_6H_3CO_2.CH_2C_6H_4NO_2$, m. 142.0°;
Phenylpropiolate, $C_6H_5C:CCO_2.CH_2C_6H_4NO_2$, m. 83°;
Thiocyanate, $CNS.CH_2C_6H_4NO_2$, m. 85°;
Oxalate $(.CO_2.CH_2C_6H_4NO_2)_2$, m. 204°;
Malonate, $CH_2(CO_2.CH_2C_6H_4NO_2)_2$, m. 85.5°;
Tartrate, $(.HOCHCO_2.CH_2C_6H_4NO_2)_2$, m. 163°;
Citrate, $C_3H_5O\text{-}(CO_2.CH_2C_6H_4NO_2)_3$, m. 102°.

BALTIMORE, MD.

[CONTRIBUTION FROM THE LABORATORY OF FOOD CONTROL, BUREAU OF CHEMISTRY, DEPARTMENT OF AGRICULTURE.]

THE IDENTIFICATION AND ESTIMATION OF LACTIC ACID IN BIOLOGICAL PRODUCTS. FIRST PAPER.[1]

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It is very obvious that there is need for a method by which the identification of lactic acid and its estimation quantitatively can be accomplished. The fact that no salts of lactic acid insoluble in water have been discovered is, perhaps, the chief cause of the absence of such a method. This paper records some of the results obtained in the search for such a method, studying, in particular, the solubility of various salts and derivatives of lactic acid in organic solvents for the purpose of determining lactic acid in biological products whether natural or fermented.

Irvine[2] resolved racemic lactic acid into its optically active components by means of the difference in solubility of their morphine salts in

[1] Read at the 20th annual meeting of the Association of American Dairy, Food and Drug Officials, Detroit, Michigan, August 5–11, 1916. Published by permission of the Secretary of Agriculture.

[2] Irvine, *J. Chem. Soc.*, **89**, 935 (1906).

water, and Lindet[1] found the difference in solubility in methyl alcohol of the acid quinine salts of citric and malic acids such that partial separation of these acids was possible through these salts.

Preparation of Materials.—Pure lactic acid consisting of the racemic acid with a small excess of *l*-acid was obtained by hydrolysis of the ethyl esters. Lactic acid of commerce, presumably prepared from fermentation products, was freed from water as completely as possible by heating under diminished pressure in a water bath at 80°. The lactic acid was esterified with absolute ethyl alcohol containing in solution dry hydrochloric acid gas in presence of zinc chloride, as the second catalyzer, according to the method of Phelps and Phelps[2] for the esterification of succinic acid. The fraction, freed under diminished pressure from water and alcohol, after drying over fused sodium sulfate, was distilled at 67° to 71° under 30 mm. pressure. Under atmospheric pressure, a portion was found to boil at 153.8° to 154.3°, showing no higher boiling portion. It was hydrolyzed by diluting with water in large volume and boiling for four hours under a return condenser. This water solution of lactic acid was extracted with ether in a continuous liquid extractor. As soon as most of the ether was removed from the ethereal solution under diminished pressure, absolute alcohol was added to the mixture and the whole heated on the steam bath to remove the last traces of the ether. The strength of the alcoholic solution was estimated by heating it on the steam bath with barium hydroxide of known strength, approximately 0.1 *N*, in excess in the presence of phenolphthalein to hydrolyze any remaining traces of ester or anhydride. Hydrochloric acid solution of known strength was added in excess to dissolve the barium carbonate present before the whole was boiled to free from carbon dioxide, a current of air freed from carbon dioxide being allowed to pass through the liquid during the boiling. The excess of hydrochloric acid was found by adding a solution of barium hydroxide of known strength.

The racemic lactic acid used in the experiments in Table III and Expts. 1, 4 and 5 of Table V was prepared by heating for some time commercial lactic acid and zinc carbonate in excess, filtering and collecting the zinc lactate which crystallized on cooling. After recrystallizing the zinc lactate from water, the zinc was removed by hydrogen sulfide, hydrogen sulfide in the clear filtrate being removed by boiling. The lactic acid was recovered from the water solution as given above for the mixture of lactic acids.

Dextrolactic acid was prepared pure from commercial sarcolactic acid by heating with zinc carbonate. The zinc *d*-lactate obtained from the evaporated solution was recrystallized from hot water. Determinations

[1] Lindet, *Compt. rend.*, **122**, 1135 (1896).

[2] Phelps and Phelps, *Am. J. Sci.*, [4] **24**, 194 (1907).

of the optical rotation of the zinc *d*-lactate in a 2 dm. tube at 20° were made with the results recorded in Table I, where they are compared with the results of previous investigators. The zinc *d*-lactate was converted to *d*-lactic acid in the manner described above for the other preparations of lactic acid.

TABLE I.—OPTICAL ROTATION OF ZINC *d*-LACTATE AND ZINC *l*-LACTATE.

	Anhydrous zinc *d*-lactate in 100 cc. (g.).	$[\alpha]_D$.	Anhydrous zinc *l*-lactate in 100 cc. (g).	$[\alpha]_D$.
Phelps & Palmer	6.9346	−6.50°	3.4672	+7.70°
	3.4673	−7.70°		
Hoppe-Seyler & Araki[1]	6.5313	−6.84°	3.3375	+7.51°
	4.1830	−7.55°		
Wislicenus[2]	6.51	−7.83°		
	4.58	−8.73°		
Irvine[3]	6.1874	−7.84°	4.9013	+7.85°
Schardinger[4]			4.85	+7.23°
Purdie[5]	6.3956	−7.74°	6.519	+7.82°
Purdie & Walker[6]	5.8807	−7.26°		
Jungfleisch & Godchot[7]	5.0	−6.0°		
	2.5	−8.0°		

Laevolactic acid was prepared, as described by Irvine,[8] by the resolution of racemic lactic acid by means of the difference in solubility of the morphine salts in water. The more difficultly soluble morphine *l*-lactate, which separated first, was treated in water solution with sodium bicarbonate to precipitate the morphine. The filtrate from the morphine was acidified with dilute sulfuric acid and extracted with washed ether in a continuous liquid extractor. The ether was evaporated, the residue dissolved in water and boiled with zinc carbonate. The filtrate from the excess of zinc carbonate was evaporated to crystallization of the zinc *l*-lactate. A determination of the optical rotation in a 2 dm. tube at 20° gave the results recorded in Table I. Laevolactic acid was prepared from the zinc salt as described above for the other preparations of lactic acid.

The lactic acid used in Expts. 5 to 10, inclusive, of Table VI was prepared from ethyl lactate which contained an excess of the ethyl *l*-lactate, as shown by its specific rotation, which was found to be $[\alpha]_D = +6.08°$ at 20°. The specific rotation of pure ethyl *l*-lactate is given by Walker[9]

[1] Hoppe-Seyler & Araki, *Z. physiol. Chem.*, **20**, 365 (1895).
[2] Wislicenus, *Ann.*, **167**, 332 (1873).
[3] Irvine, *J. Chem. Soc.*, **89**, 935 (1906).
[4] Schardinger, *Monatsh. Chem.*, **11**, 545 (1890).
[5] Purdie, *J. Chem. Soc.*, **63**, 1143 (1893).
[6] Purdie and Walker, *Ibid.*, **61**, 762 (1892).
[7] Jungfleisch and Godchot, *Compt. rend.*, **140**, 719 (1905).
[8] *Loc. cit.*
[9] *J. Chem. Soc.*, **67**, 916 (1895).

as $[\alpha]_D^{19°} = +14.52°$ and by Klimenko[1] as $[\alpha]_D = +14.19°$. It is noted that the melting points of the quinine lactate obtained in these experiments are slightly higher than the melting point of pure racemic quinine lactate as given in Table II, in all probability due to the admixture of an excess of the laevo-form.

Quinine sulfate was prepared by heating an excess of the quinine of commerce with sulfuric acid to obtain the neutral salt. This was crystallized from water, and after drying in a vacuum desiccator, was twice recrystallized from 95% alcohol. Pure quinine was obtained from the quinine sulfate, prepared as above, by precipitating with sodium hydroxide and shaking with chloroform. By evaporation of the chloroform, the quinine was obtained in an amorphous condition.

. Racemic quinine lactate was prepared by adding the molecular quantity of quinine in alcoholic solution to a solution of racemic lactic acid, and evaporating the solution to dryness under diminished pressure. The dry residue was recrystallized from hot ethyl acetate free from alcohol, the crystals being dried over potassium hydroxide in a desiccator. The quinine lactate so obtained had a melting point of 165.5° (uncorr.), melting with decomposition. The preparation used showed the following optical properties:[2] Crystallized in fine needles, extinction parallel; elongation positive; refractive indices: γ about 1.655, and α about 1.608; birefringence fairly strong, about 0.045. The individual needles show almost invariably the same two refractive indices. This is good evidence that the substance is uniaxial; the crystal system therefore either hexagonal or tetragonal.

Quinine *d*-lactate, prepared in a similar manner from quinine and *d*-lactic acid, had a melting point of 175.0° (uncorr.). The preparation used showed the following optical properties:[2] Crystallizes in long hexagonal needles and prisms, measuring up to 0.2 mm. in length and 0.01 mm. in width, and terminated by relatively flat faces, apparently rhombohedra. The needles show parallel extinction and positive elongation; the interference figure obtained on the prism is that of the optic normal of an optically positive crystal. The refractive indices are: γ about 1.660 and α about 1.588; birefringence strong, about 0.070. Practically all the needles show the same high and low refractive indices. This indicates a uniaxial crystal which, in conjunction with the hexagonal outline of the cross-section of the needles is good evidence that the substance is hexagonal.

[1] *J. Russ. Phys. Chem. Soc.*, **12**, 30 (1880).

[2] The statements of the crystallographic properties of this salt and of quinine *d*-lactate, of quinine *l*-lactate and of guanidine lactate are furnished by Dr. Fred E. Wright of the Geophysical Laboratory of the Carnegie Institution of Washington. The grateful appreciation of the authors is here acknowledged. See Table I on page 138.

Quinine *l*-lactate, prepared in a similar manner from quinine and *l*-lactic acid, had a melting point of 171.0° (uncorr.). This preparation[1] is less coarsely crystalline than the quinine *d*-lactate, otherwise the optical properties are practically identical. The refractive indices are approximately: $\gamma = 1.660$; $\alpha = 1.588$; extinction parallel; elongation positive; birefringence strong; optical character positive; crystal system probably hexagonal. It would be difficult to distinguish the quinine *d*-lactate and the quinine *l*-lactate on a basis of their optical properties alone.

The other salts of quinine used in the experiments recorded in Table II were prepared by adding molecular quantities of quinine in alcoholic solution to the commercially pure acids, separately, before evaporating under diminished pressure.

Guanidine sulfate was purified from commercially pure guanidine sulfate by precipitation from water solution by the addition of alcohol and drying in a desiccator over potassium hydroxide.

Guanidine lactate was prepared from molecular quantities of racemic lactic acid and guanidine carbonate which had been recrystallized from commercially pure guanidine carbonate by the addition of alcohol to the water solution and was dried as above. The guanidine racemic lactate so obtained had a melting point of 161.5–162.0° (uncorr.). This preparation showed the following optical properties:[1] Individual grains commonly aggregated in fine clusters; scattered through the powder are minute tabular crystals, usually of rectangular outline. Refractive indices approximately $\alpha = 1.510$; $\gamma = 1.600$; birefringence strong; optic axial angle large; optical character apparently positive. The crystals do not extinguish uniformly and probably belong to either the monoclinic or the triclinic system.

The solvents were purified as indicated in Table II, the carbon tetrachloride being the commercially pure material.

TABLE II.—MELTING POINTS AND APPROXIMATE SOLUBILITY OF QUININE SALTS.

	m. p. (uncorr.).	Solubility.			
		CCl_4.	$CHCl_3$ (alcohol-free).	Ethyl acetate (alcohol-free). Cold.	Ethyl acetate (alcohol-free). Hot.
Quinine racemic lactate	165.5°	1 in 14,000	1 in 3.5	1 in 350	1 in 30
Quinine *d*-lactate	175.0°	1 in 9,000		1 in 400	
Quinine *l*-lactate	171.0°	1 in 21,000		1 in 500	
Quinine formate	110–113°	1 in 16,000			
Quinine acetate	124–126°	1 in 2,000	Easily soluble		
Quinine propionate	110–111°	1 in 450	Easily soluble		
Quinine butyrate	77.5°	1 in 25	Easily soluble		
Quinine succinate	192.0°	1 in 100,000			1 in 250
Quinine tartrate	202.5°	1 in 250,000			1 in 3,000
Quinine malate	177.5°	1 in 125,000			1 in 200
Quinine citrate	183.5°	1 in 60,000			1 in 1,200
Quinine sulfate	214.0°	1 in 40,000	1 in 3000	1 in 14,000	1 in 7,500
Quintoxine lactate		1 in 900			

[1] See foot-note 2 on page 139.

The solubilities of various salts of lactic, formic, acetic, propionic, butyric and some other acids, especially those of organic bases, were studied in various organic solvents. The solubilities of a few of these salts in some of the common solvents were approximately determined, and are recorded in Table II, together with their melting points. These solubility determinations were made after each quinine salt had, in some cases, been boiled for eighteen hours under a return condenser, filtering immediately, or had, in other cases, stood in contact with a definite volume of the solvent in a stoppered flask for periods of seventy-two hours, being shaken at frequent intervals, filtering, evaporating the solution to dryness and weighing the residue, after drying in a jacketed vacuum oven at 50°.

It is seen from the results recorded in Table II that a fair separation of the quinine lactate from the propionate and butyrate could be made by the difference in solubility in carbon tetrachloride, but no separation is possible of the lactate, formate and acetate as the difference in solubility is insufficient. Lactic acid was separated from formic and acetic acids by conversion of the three acids to their ethyl esters. As the boiling point of ethyl lactate is 154.5°, of ethyl acetate 77° and of ethyl formate 54.4°, the formic and acetic esters were removed by fractional distillation. Ethyl lactate was separated from ethyl citrate in this way as its boiling point is 294°. Ethyl lactate probably could be separated from ethyl tartrate which boils at 280°.

Estimation of Lactic Acid as Guanidine Lactate.

In the preliminary experiments which were made to determine the conditions under which small quantities of lactic acid could be quantitatively converted to ethyl lactate and also in the experiments which were made to determine the conditions under which the ethyl lactate could be separated from ethyl acetate by fractional distillation, the yields of ethyl lactate were determined by converting to the guanidine lactate and weighing. The lactic acid was esterified following the procedure outlined by Phelps and Phelps[1] for succinic acid. As the amount of lactic acid to be esterified was very small vaseline was put into the second flask to hold the lactic acid until it might be exposed to the action of the vapors of alcohol as they passed through the liquid. The lactic acid was esterified, as shown in Fig. 1, in an apparatus consisting of a side neck Flask B of 75 cc. capacity, connected with a condenser, carrying a thermometer and from Flask A an inlet tube adjusted to dip beneath the liquid to the same depth as the thermometer. Flask A carried a thistle tube curved at its lower end to prevent egress of alcoholic vapors and, also, the exit tube to flask B. In Flask B the lactic acid was heated to 100° by means of a glycerol bath, with ten cubic centimeters of the total amount

[1] *Loc. cit.*

of absolute alcohol containing in solution dry hydrochloric acid gas with the vaseline and zinc chloride, while from Flask A the alcohol containing in solution hydrochloric acid gas was driven into the mixture in B, where esterification took place. The temperature of the vaseline mixture in Flask A during the course of the esterification and the time of the esterification are indicated in Table III for each determination, the temperature being kept between 100° and 110°. This could be easily regulated by keeping the temperature of the glycerol bath 10 or 15° higher. Precipitated silver carbonate in suspension in absolute alcohol was added to the distillate with stirring in order to remove the hydrochloric acid as the insoluble silver chloride. The green silver carbonate tending to become brown on exposure to the light was quickly converted to the white silver chloride and the point at which sufficient silver carbonate had been

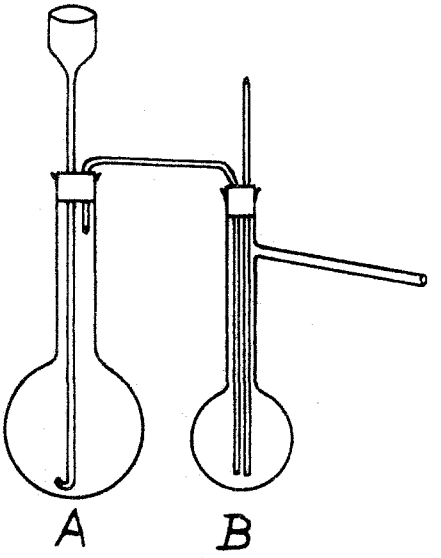

Fig. 1.

TABLE III.—ESTERIFICATION OF LACTIC ACID AND ESTIMATION AS GUANIDINE LACTATE.
(Temperature of esterification 100–110°.)

Zinc lactate taken, g.	Lactic acid taken, g.	Guanidine lactate found, g.	Lactic acid found, g.	Per cent. yield.	Absolute alcohol + HCl used, cc.	Time of esterification. Min.	Melting point of guanidine lactate.
	0.0929	0.1435	0.0866	93.3	100	30	
0.1989		0.1960	0.1184	92.4	100	40	
	0.1858	0.2755	0.1664	89.6	100	30	160.0° to 160.5°
	0.2270	0.3228	0.1949	85.9	100	35	160.5°
	0.1644	0.2605	0.1573	95.7	200	75	160.0° to 160.5°
	0.1644	0.2724	0.1645	100.0	200	120	159.0°

added to completely convert the hydrochloric acid to silver chloride could be easily told by the fact that the precipitate remained greenish yellow or brown as soon as an excess of silver carbonate was present. The precipitated silver carbonate, to avoid the reducing action of the alcohol on standing, was kept in suspension in water in brown glass bottles, and decanted with absolute alcohol just before using. The solution containing the silver chloride and silver carbonate in suspension was allowed to stand for two or three hours in a dark place with occasional stirring to complete the conversion of hydrochloric acid to silver chloride. The precipitate was then filtered through asbestos and washed with absolute

alcohol. To the filtrate an excess of barium hydroxide solution was added, and the solution was heated on the steam bath for about an hour to complete the hydrolysis of the ethyl lactate. During this interval the solution was tested from time to time with a strip of filter paper an eighth of an inch wide which was prepared for use by dipping in phenolphthalein solution and drying, in order to determine if barium hydroxide was still present in excess. If the paper remained colorless, enough barium hydroxide solution was added, so that at the end of heating barium hydroxide remained in excess. After passing a current of carbon dioxide sufficiently long to precipitate the excess of barium hydroxide the solution was evaporated to dryness. The dry residue was extracted with 25 cc. of water and filtered on asbestos. To the filtrate containing the barium lactate, an excess of neutral guanidine sulfate in solution in water was added, to complete precipitation of the barium as barium sulfate. The solution, containing the precipitated barium sulfate, was allowed to stand until the precipitate had subsided, when it was filtered on asbestos in a perforated porcelain crucible and the precipitate washed with distilled water. The filtrate, contained in an Erlenmeyer flask, was evaporated nearly to dryness by distillation, the evaporation being completed on the steam bath, the last trace of moisture from the flask being removed by allowing a gentle current of air to pass over the solution from a glass tube suspended in the flask. After it had evaporated completely to dryness, 50 cc. of absolute alcohol were added, the flask stoppered and allowed to stand eighteen hours, shaking occasionally, to dissolve the guanidine lactate. The solution was filtered and the residue washed with absolute alcohol. The proportion of guanidine sulfate dissolved is shown in Table IV, which gives the solubilities in absolute alcohol of guanidine lactate and guanidine sulfate. To insure removal of all of the guanidine lactate a second extraction was always made. The combined filtrates, containing the guanidine lactate, were evaporated to dryness, the residue dried in a jacketed vacuum oven at 50° and weighed. The melting points of the residues, as given in Table III and Table V, were determined and were generally slightly low, owing to the admixture of a slight amount of guanidine sulfate which was dissolved by the absolute alcohol. It was found that under the proper conditions, by distilling a sufficient quantity of alcohol containing dry hydrochloric acid gas in solution all of the lactic acid could be converted to the ester and collected as such in the distillate. In Expts. 1 to 4, inclusive, of Table III, in which 100 cc. of the absolute alcohol containing hydrochloric acid were used in the esterification, yields varying from 85.9 to 93.3% were obtained, the highest percentage

TABLE IV.—APPROXIMATE SOLUBILITY IN ABSOLUTE ALCOHOL.

Guanidine lactate	1 pt. in 130
Guanidine sulfate	1 pt. in 10,870

being obtained when the smallest amounts of lactic acid were present, and the lowest percentage being obtained in the experiments in which the larger quantities of lactic acid were present. This seemed to point to the fact that the quantity of the absolute alcohol mixture used was not sufficient to convert the lactic acid to ester and collect it in the distillate. That this was the case is shown in Expts. 5 and 6 of Table III, where 200 cc. of the absolute alcohol mixture were used in the esterification, giving yields of 95.7 and 100.0%.

In the following manner it was found possible to separate lactic and acetic acids as esters. Lactic acid in definite amount, as shown in Table V, alone in some cases, in the presence of acetic acid, in others, was esterified in the esterification apparatus under the conditions just described, the distillate being collected in a 300 cc. round-bottom flask. Through a Hempel fractionating column 1.8 cm. in diameter filled with glass beads to a length of 12 cm., the liquid was distilled to a volume of 20 cc. Then, while still boiling, through the Hempel column 90 cc. of absolute alcohol were added and the solution again distilled to a volume of 20 cc. By addition of a second portion of 90 cc. of absolute alcohol and distilling to 20 cc. the last traces of ethyl acetate were removed. One hundred cubic centimeters of absolute alcohol were poured through the column containing the glass beads to remove any traces of ethyl lactate and the solution was treated as described above for the conversion of the ethyl lactate to guanidine lactate. The experiments recorded in Table V show that with a fractionating column of the above dimensions and distillation of 100 cc. of alcohol in an hour all of the ethyl acetate may be distilled with only a slight loss of ethyl lactate. In the case of Expt. 2, in which only two distillations were made, probably some acetic acid remained in the residue, as shown by the low melting point of the guanidine lactate, but in the case of Expts. 3 and 5 in each of which three distillations were made, the acetic acid seems to have been removed. These results show amounts of guanidine lactate less than theory for the same reason as the results of Expts. 1 and 4, inclusive, of Table III, because the 100 cc. of absolute alcohol are insufficient in amount to collect the lactic and acetic esters in the distillate but they serve to indicate that there is only a slight further loss of lactic acid in the fractional distillation, as is evident when they are compared with the results recorded in Table III, where results of esterification only are recorded. This was definitely proved in the case of Expts. 7 and 8 of Table V, in which the total combined distillates from the fractionation were treated with silver carbonate to remove the hydrochloric acid and the filtrate from this precipitate hydrolyzed with an excess of barium hydroxide. The excess of barium hydroxide was removed by carbon dioxide, the solution evaporated to dryness, and the residue extracted with a little water. The filtrate was

TABLE V.—SEPARATION OF LACTIC AND ACETIC ACIDS AND ESTIMATION OF LACTIC ACID AS GUANIDINE LACTATE.

Zinc lactate taken, g.	Lactic acid taken, g.	Lactic acid found, g.	Per cent. yield.	Acetic acid present, g.	Time of distillation. Minutes.	Vol. of residue, cc.	Abs. alc. added in distillations, cc.	M. p. of guanidine lactate.
	0.1135	0.0957	84.3		120	20	90	159.5°
					130	20		
0.2008		0.1140	88.1	0.1030	85	10 to 15	90	157.5°
					70	17		
0.4001		0.2297	89.1	0.1030	60	25		
					130	25	90	160.0–160.5°
					45	25	45	
	0.2270	0.1997	88.0		120	20		
					115	17	90	160.0°
					110	20	45	
	0.2270	0.1963	86.5	0.1030	50	20		
					75	20	90	160.0°
					20	20	45	
	0.0822	0.0737	89.6		65	20		
					55	20	90	160.0°
					50	20	90	
	0.1644	0.1309	79.6		50	15		
					40	20	90	
					55	20	90	
	0.2466	0.1865	75.6		65	20		
					50	20	90	
					50	20	90	

evaporated to dryness and the residue of barium lactate weighed. These weights, calculated to percentage of the lactic acid originally present showed that about 2% of lactic acid had been lost in the fractionation. In later experiments a larger fractionating column was used, the column of glass beads being 15 cm. and diameter 2.5 cm. It was found that the amount of ethyl lactate which would distil, using a fractionating column of this size, is negligible. This method of determination as guanidine lactate would not be applicable in presence of acids whose esters have boiling points near that of lactic ester as these would distil with the lactic acid in the esterification and would not be entirely removed in the fractional distillation, and so would appear in the final product as the guanidine salts along with the guanidine lactate. Determinations were made of the solubilities of guanidine lactate, guanidine butyrate, guanidine propionate, and also of guanidine acetate and guanidine formate, in water, absolute alcohol, ether, acetone, ethyl acetate freed from alcohol, carbon tetrachloride, and benzene. All of these salts were found to be rather insoluble in the solvents mentioned excepting water and absolute alcohol, and, in these they were all soluble to a greater or less extent, so that a separation could not be effected.

This method for the determination of lactic acid as guanidine lactate was used to study the conditions under which lactic acid could be quantitatively converted to its ethyl ester and also the conditions under which the ethyl lactate so obtained is separated by fractional distillation from ethyl acetate and from ethyl formate. Further, it served as a convenient method for the estimation and for the determination of the purity of the product, because of the crystalline form and well-defined melting point of the guanidine lactate.

Estimation of Lactic Acid as Quinine Lactate.—Lactic acid was estimated as quinine lactate, because in this way a separation of the lactic acid from propionic and butyric acids could be made by means of the differences in solubility of the quinine salts in carbon tetrachloride, as well as a separation from formic and acetic acids on fractional distillation of the ethyl esters, and also a separation from acids with high boiling esters, such as citric and tartaric acids.

The material containing lactic acid was esterified in the apparatus as above described, with 1 gram of zinc chloride, 25 cc. of vaseline, and 200 cc. of absolute alcohol, containing in solution 2.5 g. of dry hydrochloric acid gas per liter. The temperature in the esterification flask was kept between 100° and 110° during the esterification which was concluded in about an hour and a half. The distillate, contained in a round-bottom flask, was then fractionated through a Hempel column filled with glass beads, the length of the column of glass beads being 15 cm., the width 2.5 cm. The liquid was distilled to a volume of 20 cc., 100 cc. of absolute alcohol was added through the top of the fractionating column and the liquid was distilled again to a volume of 20 cc.; a second portion of 100 cc. of absolute alcohol was added and the distillation to a volume of 20 cc. was repeated. The column of glass beads was then thoroughly washed with absolute alcohol into the residue in the flask. This was treated with an excess of silver carbonate in the manner previously described, allowed to stand two hours in the dark before filtering on asbestos. To the filtrate was added an excess of barium hydroxide solution and the solution heated on the steam bath, with addition of more barium hydroxide if necessary, until the solution remained pink with phenolphthalein after heating one hour, showing complete hydrolysis of the ethyl lactate to barium lactate. A current of carbon dioxide was passed through the solution to precipitate the excess of barium hydroxide as carbonate and the solution evaporated to dryness. The residue was extracted with 25 cc. of water and filtered through an asbestos filter. To the filtrate was added a slight excess of a solution of neutral quinine sulfate in hot water to complete precipitation of the barium sulfate. The solution was cooled in ice water, filtered as soon as the precipitate of barium sulfate had subsided. The quinine sulfate required could be calculated approximately

from the quantity of solution of barium hydroxide used. The filtrate, containing the lactic acid as quinine lactate with any excess of quinine sulfate, was rinsed into a side neck distilling flask with 95% alcohol, which served to prevent foaming during distillation, and evaporated to dryness by distilling under diminished pressure, preferably as low as 15 mm., to keep the temperature of the solution as low as may be. After the distillation was completed, the residue which had collected on the sides of the flask was removed with 10 cc. of alcohol, and the alcohol removed by distillation under diminished pressure. In this way all of the moisture was removed, the residue being left in condition to be more readily brought in contact with the solvent in the subsequent treatment with carbon tetrachloride. The residue was warmed with 50 cc. of carbon tetrachloride to remove any quinine propionate or quinine butyrate which might be present, the amount of carbon tetrachloride necessary being shown in Table II by the solubilities of the propionate and butyrate. But the more carbon tetrachloride used, the greater will be the amount of quinine lactate dissolved, so that if the propionate and butyrate are present in large amounts relative to the lactate, a greater loss of lactic acid will be entailed than if they are present in smaller amounts. The carbon tetrachloride was removed by filtration after standing eighteen hours and the residue washed with carbon tetrachloride. The residue left in the flask was freed by a gentle current of air from the carbon tetrachloride, and together with the residue on the filter paper was allowed to stand for an hour with chloroform free from alcohol to dissolve the quinine lactate and separate it from the excess of quinine sulfate. It is necessary to use chloroform free from alcohol, as the solubility of quinine sulfate in chloroform is greatly increased by the presence of alcohol. The chloroform solution of quinine lactate which was obtained by removing the quinine sulfate by filtration and washing with alcohol-free chloroform was carefully evaporated to dryness on the steam bath and dried in the vacuum oven at 75° and weighed. The quinine lactate obtained by

TABLE VI.—SEPARATION OF LACTIC ACID AND ESTIMATION OF LACTIC ACID AS QUININE LACTATE.

Lactic acid present, g.	Gram acid present.					Quinine lactate found, g.	Lactic acid found, g.	Per cent. yield.	Melting point of quinine lactate.
	Formic.	Acetic.	Propionic.	Butyric.	Citric.				
0.0821	.....	.....	.....	.....	.....	0.3749	0.0815	99.2	165.0°
0.1642	.....	.....	.....	.....	.....	0.7437	0.1616	98.4	..
0.0821	.....	0.1032	.....	.....	.....	0.3840	0.0834	101.6	165.0° to 165.5°
0.0821	.....	.....	0.0503	.....	.....	0.3671	0.0798	97.2	165.5°
0.2786	.....	.....	.....	.....	.....	1.2492	0.2714	97.4	166.5°
0.1393	0.1054	0.1032	.....	.....	.....	0.6342	0.1378	98.9	165.0° to 165.5°
0.1393	.....	.....	.....	0.0534	.....	0.6163	0.1339	96.1	166.5°
0.1393	0.0527	0.0516	0.0503	0.0446	.....	0.6135	0.1333	95.7	..
0.1393	.....	.....	.....	.....	0.2000	0.6217	0.1351	97.0	165.0°
0.5572	.....	.....	.....	.....	.....	2.5462	0.5533	99.3	166.5°

evaporation of the chloroform solution sometimes appeared in a noncrystalline, amorphous form, easily convertible to the crystalline form by warming in pure ethyl acetate free from alcohol and cooling. This also served to remove the slight amount of quinine sulfate which was dissolved by the chloroform. It was filtered from the hot ethyl acetate solution and its weight deducted from the weight of the quinine lactate found. The quinine lactate which crystallized from the ethyl acetate was filtered and identified by its melting point.

Quinotoxine and the Effect of Its Formation.—H. C. Biddle[1] has shown that when quinine is heated in the form of its salts in aqueous solution, either with or without excess of acid, it is converted to its poisonous isomer, quinotoxine, which has also been described by Von Müller and Rohde,[2] and shown by them to be identical with the quinicine which was originally described by Pasteur[3] in 1853. Biddle has further shown that the rate of conversion in general increases with acids of decreasing dissociation constant. Quinine salts of strong acids, such as hydrochloric for instance, showed no detectable conversion on heating to 98–102° for 48 hours. But on heating quinine with an excess of weak acids, such as acetic or lactic, for example, to 98–102° for 48 hours, Biddle obtained almost quantitative conversion to quinotoxine, while at 36° he found that appreciable conversion took place, although at a much slower rate, approximately 2% in 48 hours.

In order to investigate what would be the effect in this method of the formation of quinotoxine lactate from the quinine lactate, some pure quinotoxine was prepared according to the directions given by Biddle. Quinotoxine lactate was obtained from the pure quinotoxine as a thick, pasty, noncrystalline mass, and its solubility in carbon tetrachloride was found to be about 1 part in 900. As this solubility is much greater than that of quinine lactate in carbon tetrachloride, it is seen that any formation of quinotoxine lactate would involve the loss of that amount of lactic acid in an estimation of lactic acid by this method. For this reason it is essential that the aqueous solutions of quinine lactate should not be allowed to stand and, especially, that they should not be heated but should be evaporated by distillation under diminished pressure, preferably as low as 15 mm., to prevent the formation of quinotoxine lactate in appreciable quantity. The results of the estimation of lactic acid in the presence of other acids by the method as described are given in Table VI.

From the results here recorded it is evident that lactic acid in the proportions indicated in Table VI can be identified and estimated alone in

[1] Biddle, THIS JOURNAL, **34**, 500 (1910).

[2] Von Müller and Rohde, *Ber.*, **33**, 3214 (1900).

[3] Pasteur, *Compt. rend.*, **37**, 110 and 166 (1853).

solutions, as shown in Expts. 1, 2, 5 and 10. Or, before estimation the lactic acid can be separated from acetic acid, as shown in Expt. 3. Or, it can be separated from formic and acetic acids, as shown in Expt. 6; or, from citric acid, as shown in Expt. 9; or, from propionic acid, as shown in Expt. 4; or, from butyric acid, as shown in Expt. 7; or, from formic, acetic, propionic and butyric acids, as shown in Expt. 8.

Summary.

It has been shown that lactic acid may be estimated as guanidine lactate, identified by its melting point after separation by esterification from citric and tartaric acids and by fractional distillation from formic and acetic acids. Further, it has been shown that lactic acid may be separated from mixtures containing formic, acetic, propionic, butyric and citric acids, and accurately estimated by weighing as quinine lactate, which may be identified by determination of its melting point.

The separation from citric acid and other acids whose ethyl esters also have high boiling points is effected by esterification with the vapor of alcohol, containing dry hydrochloric acid gas in solution passed through the mixture suspended in vaseline at a temperature of 100–110°, using zinc chloride as a second catalyzer; the ethyl lactate passes quantitatively into the distillate, while the ethyl citrate remains in the flask. By fractional distillation of the distillate through a Hempel fractionating column filled with glass beads the ethyl formate and ethyl acetate together with a large part of the ethyl propionate and ethyl butyrate are removed. The residue in the flask, containing the ethyl lactate, is hydrolyzed and converted to the quinine salts and the quinine lactate is separated from the propionate and butyrate by the solubility of the quinine salts of the latter in carbon tetrachloride. The quinine lactate may then be weighed and identified by its melting point.

WASHINGTON, D. C.

CARBON MONOXIDE, OCCURRENCE FREE IN KELP.[1]

(Nereocystis luetkeana.)

BY SETH C. LANGDON.

Received October 9, 1916.

Studies have been made by Rosanoff,[2] Willie,[3] and Lucas,[4] on the gases contained in the floaters which buoy up certain marine plants. Their results show an oxygen-nitrogen mixture, the percentage composition of which varies with different plants and conditions. During the summer of

[1] A more detailed account of this work with extension to other gas-bearing algae is to be printed in the publications of the Puget Sound Marine Station.

[2] *Mem. Soc. Imp. Sci, Nat. Cherbourg*, 13, 143–240 (1868).

[3] Just's *Bot. Jahresber.*, 17, 226 (1899); *Chem. Zentr.*, 1890, I, 1006.

[4] *Proc. Linn Soc., New South Wales*, 36, 626–631 (1911).