

tion of 3 mole equivalents of hydrogen. Filtration and evaporation of the solution then gave 88% of 1,2-hexahydrobenzylidene-D-glucofuranose, m.p., after recrystallization from water, 179–180°; $[\alpha]^{25}_D$ 10.2 in pyridine, *c* 2. *Anal.* Calcd. for $C_{13}H_{22}O_6$: C, 56.9; H, 8.09. Found: C, 57.1; H, 8.07.

Hydrogenolysis of 1,2-Benzylidene-D-glucofuranose.—A sample of 0.2 g. of II in 25 ml. of absolute ethanol was shaken with hydrogen at room temperature and atmospheric pressure in the presence of 0.2 g. of "S-palladium black."¹² Hydrogenolysis was complete in 1.5 hours with the absorption of 2.5 mole equivalents of hydrogen. After filtration, the solution showed $[\alpha]^{25}_D$ 64°, calculated for D-glucose. For the equilibrium rotation of D-glucose in absolute ethanol, Rowley¹³ reports $[\alpha]^{25}_D$ 66.5°.

α - and β -Tribenzoates of 4,6-Benzylidene-D-glucose.—The benzylation of I with benzoyl chloride and pyridine was carried out according to the directions of Brigg and

Grüner,⁶ to give 95% of crude, crystalline product. Fractional recrystallization from ethanol gave 1,2,3-tribenzoyl-4,6-benzylidene- β -D-glucose,⁶ m.p. 193°; $[\alpha]^{30}_D$ -10.6° in chloroform, *c* 1.3 and 1,2,3-tribenzoyl-4,6-benzylidene- α -D-glucose, m.p. 166–167°; $[\alpha]^{25}_D$ 47.4° in chloroform, *c* 1.3. *Anal.* (α -anomer). Calcd. for $C_{34}H_{28}O_9$: C, 70.3; H, 4.86. Found: C, 70.5; H, 4.81.

The separation of the anomers by fractional crystallization was accompanied by considerable loss of material and it was not possible to estimate the relative proportions of the two products in the mixture.

1,2-Benzylidene-3,5,6-tribenzoyl-D-glucose.—The benzylation of II with benzoyl chloride and pyridine at room temperature yielded 98% of the crystalline tribenzoate. Recrystallization from ethanol and then from acetone by the addition of water gave pure 1,2-benzylidene-3,5,6-tribenzoyl-D-glucose, m.p. 107–108°; $[\alpha]^{25}_D$ -62.7° in chloroform, *c* 1.4. *Anal.* Calcd. for $C_{34}H_{28}O_9$: C, 70.3; H, 4.86. Found: C, 70.2; H, 4.58.

(12) V. K. Kändler, E. Schärfe and P. Henrich, *Ann.*, **565**, 51 (1949).

(13) H. H. Rowley, *THIS JOURNAL*, **62**, 2563 (1940).

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Bitter Principles in Citrus. III. Some Reactions of Limonin¹

BY OLIVER H. EMERSON

The hydrogenation of limonin ($C_{26}H_{30}O_8$) gives a mixture of tetrahydrolimonin ($C_{26}H_{34}O_8$) and hexahydrolimoninic acid ($C_{26}H_{38}O_8$). Limonin appears to be bicarboyclic and has two ethylenic links, one of which forms an allylic system with the potential hydroxyl of a lactone group. The oxidation of limonin with alkaline hypiodite gives limonic acid ($C_{26}H_{30}O_8$). Tetrahydrolimonin and hexahydrolimoninic acid behave similarly. The reaction apparently involves the opening of a lactone ring and the formation of a new carbocyclic ring. One lactone ring is opened by hydrogenolysis and the other by oxidation. Treatment of limonin with hydriodic acid gives citrolin ($C_{26}H_{22}O_6$) which appears to be tricarboyclic, and has four ethylenic links, one of which is conjugate with the carbonyl group, and one with a lactone group.

Previous investigations have shown that limonin, the bitter principle which occurs in Navel oranges and other Rutaceae plants² is a keto-dilactone of the composition $C_{26}H_{30}O_8$.^{3–5} The two lactone groups are easily opened by alkali, and recycled by acid, indicating they are γ -lactones. The three remaining oxygen atoms cannot be detected chemically and are presumed to be cyclic oxides. By fusion with potassium hydroxide, limonin yields acetone, and from the non-volatile residue, by selenium dehydrogenation, Koller and Czerny³ reported the isolation of 1,2,5-trimethylnaphthalene, while by oxidizing limonin with manganese dioxide and sulfuric acid, they obtained benzene-pentacarboxylic acid. By hydrogenation over palladium, they observed limonin to take up about three moles of hydrogen yielding a relatively small amount of a neutral tetrahydrolimonin and a larger amount of a substance which they called hexahydrolimoninic acid, whose analysis agreed with the composition $C_{26}H_{38}O_8$. However, the analysis of the methyl ester indicated the acid to be a very stable hydrate, which they were unable to dehydrate without further decomposition. The hydrogenation of limonin has been reinvestigated by

Rosenfeld and Hofmann⁶ who obtained similar results.

By oxidizing limonin in 6 *N* alkali with potassium manganate, Geissman and Tulagin⁴ obtained limonic acid which they believed to be $C_{26}H_{22}O_7$, while by treatment with hydriodic acid they obtained citrolin, $C_{26}H_{28–30}O_6$, and desoxylimonin, $C_{26}H_{30}O_7$. Unfortunately the yield of the latter substance was too small to permit further investigation. They noted that citrolin was very easily altered by alkali and gave acetone far more readily than limonin or limonic acid, but no crystalline derivatives of citrolin could be obtained.

The purpose of this communication is to extend and correlate some of these observations. By refluxing limonin for 15 to 20 minutes with a moderate excess of 0.1 *N* methanolic potassium hydroxide, both lactone groups are opened quantitatively. By removing the methanol and titrating to the half-neutralization point of each acid group, the dissociation constants can be estimated. The substance is a very strong organic acid, pK' 2.7, pK'' 4.7. The rate of recycling of the two lactone groups depends on concentration of the hydrogen ion, and at *pH* 3.0, the stronger acid group cyclizes several times as fast as the weaker group.

By hydrogenation over palladium-on-charcoal, either in ethanol containing some HCl, as done by Koller and Czerny, or in acetic acid, limonin is hydrogenated readily, although rather slowly, yielding tetrahydrolimonin and hexahydrolimoninic acid which are both mixtures of epimers. Tetra-

(1) Enzyme Research Division Contribution No. 138. Article not copyrighted.

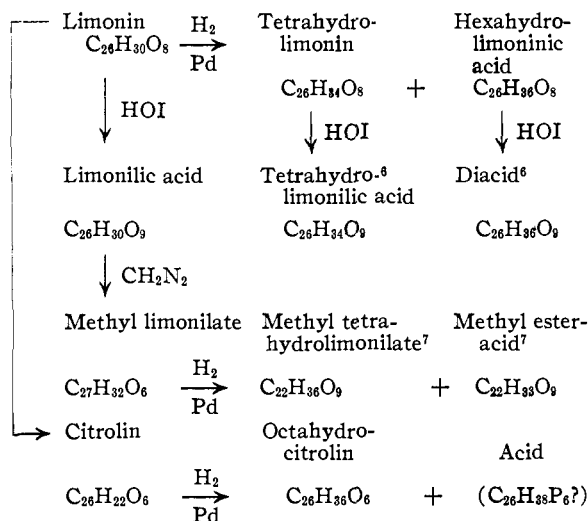
(2) M. S. Schechter and H. L. Haller, *THIS JOURNAL*, **62**, 1307 (1940).

(3) (a) G. Koller and H. Czerny, *Monatsh.*, **67**, 248 (1936) *C. A.*, **30**, 3831 (1936). (b) G. Koller and H. Czerny, *Monatsh.*, **70**, 26 (1937) *C. A.*, **31**, 4334 (1937).

(4) T. A. Geissman and V. Tulagin, *J. Org. Chem.*, **11**, 760 (1946).

(5) O. H. Emerson, *THIS JOURNAL*, **70**, 545 (1948).

(6) R. S. Rosenfeld and K. Hofmann, *ibid.*, **73**, 2491 (1951).



hydrolimonin is stable to further hydrogenation over palladium and, in contrast to limonin, gives no color with tetranitromethane and, after opening the lactone rings with alkali, reduces permanganate only very slowly. Like limonin, tetrahydrolimonin forms an oxime and gives no methane with methylmagnesium iodide,⁸ showing that the carbonyl group has not been reduced, and that no oxide rings have opened. Hence the conclusion seems justified that limonin has two ethylenic links. From the composition of the substance, and assuming the presence of two lactone groups, one carbonyl, three oxide rings, and two ethylenic links, it follows that limonin must have two carbocyclic rings.

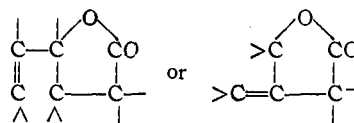
Our experience confirmed Koller and Czerny's observation that hexahydrolimoninic acid crystallizes readily only in the presence of some water and, when dried to constant weight, gave analytical values agreeing with the composition $C_{26}H_{36}O_9$. By dissolving the acid in hot chlorobenzene, then concentrating and adding small amounts of iso-octane, the acid separated amorously, but on drying to constant weight at 100° at 0.1 mm. it gave analytical values in excellent agreement with the composition $C_{26}H_{36}O_8$. That this treatment had not altered the substance was shown by the fact that it dissolved readily in sodium bicarbonate and on acidification gave crystals of the same appearance and melting point as the original hydrate. The methyl ester, obtained by treating the acid with diazomethane, crystallized beautifully from *t*-butanol. Moderate drying gave a product of the composition $C_{27}H_{38}O_8 + \frac{1}{2} H_2O$, but complete drying required several hours heating at 140° at 0.1 mm.

The hydrogenation of limonin to hexahydrolimoninic acid thus clearly involves, in addition to saturating the two ethylenic links, the hydrogenolysis of a lactone ring. Although this phenomenon is particularly characteristic of α,β -unsaturated lactones the failure of limonin to give a color with

(7) Hydrogenations in the limonin series appear prone to yield mixtures of epimers, and the relationships of these compounds were not ascertained.

(8) The author wishes to express his sincerest thanks to Professor A. J. Haagen-Smit and the late Dr. G. Oppenheimer of the California Institute of Technology for this determination.

sodium nitroprusside, decolorize bromine, or reduce Tollens reagent is not in keeping with such a compound.⁹⁻¹² The most likely explanation would appear to be that a somewhat hindered double bond exists in a system such as



making the hydrogenolysis of the lactone analogous to the hydrogenolysis of a benzyl ester.

In agreement with Koller and Czerny,⁸ we found that hexahydrolimoninic acid melts with decomposition at 165–170°, evolving carbon dioxide. The substance is a strong organic acid, *pK* 2.7, corresponding with the strong acid group of limonin.

While searching for a method for estimating limonin, it was found that the substance dissolved in a slight excess of dilute alkali reacts very easily with hypoiodite at a *pH* of about 11, reducing the equivalent of two atoms of iodine. The reaction proceeds so smoothly that, in the absence of interfering substances, it can be used very satisfactorily for estimating limonin. On acidifying the reaction mixture, limonic acid was obtained in almost quantitative yield. Obviously, if the equivalent of only two atoms of iodine is involved, limonic acid cannot have the composition $C_{25}H_{28}O_9$, but must be $C_{26}H_{30}O_9$, and our analyses of the acid, the methyl ester, and the methyl ester-oxime are in agreement with the values calculated for the latter formula.

In contrast with hexahydrolimoninic acid, limonic acid is only a moderately strong organic acid. It is readily precipitated from the solution of its salts by the addition of acetic acid. Its insolubility in water makes it impossible to titrate in that solvent, but by titrating in 50% acetone to the midpoint, using a glass electrode, the apparent *pK* was 5.7. In 50% acetone the apparent *pK* of hexahydrolimoninic acid was increased from 2.7 to 4.0, so it seems reasonable to conclude that the actual *pK* of limonic acid is of the order of 4.4. By heating limonic acid with a slight excess of dilute alkali to convert it to the diacid, *pK* values of 2.8 and 4.8 were found. Hence the acid group of limonic acid corresponds with the weaker acid group of limonin.

Limonic acid contains a free hydroxyl group shown by the band at 2.8 μ in the infrared spectrum of methyl limonilate, while limonin, tetrahydrolimonin, citrolin and octahydrocitrolin show no bands in the region of hydroxyl stretching, 2.6 to 3.0.^{13,14} With cold methylmagnesium iodide,

(9) F. A. Kuehl, Jr., R. P. Linstead and B. A. Orkin, *J. Chem. Soc.*, 2213 (1950).

(10) W. Cocker and S. Hornsby, *ibid.*, 1157 (1947).

(11) M. S. Newman and C. A. VanderWerf, *THIS JOURNAL*, **67**, 233 (1945).

(12) W. D. Paist, E. R. Blout, F. C. Uhle and R. C. Elderfield, *J. Org. Chem.*, **6**, 273 (1941).

(13) H. M. Randall, Nelson Fuson, R. G. Fowler and J. R. Dangle, "Infrared Determination of Organic Structures," D. Van Nostrand, New York, N. Y., 1949, p. 239.

(14) The author wishes to express his thanks to Dr. Fred Stitt and Mr. Glen F. Bailey for the measurement of the infrared spectra. It is expected that the data will be published shortly.

methyl limonilate gave 0.71 to 0.75 mole of methane, and the low value is readily explicable by the presence of the other groups which add Grignard reagent and render the molecule insoluble. Limonic acid is therefore a hydroxy-acid, showing that a lactone group has been opened. Since the acid has the same number of hydrogen atoms as limonin, its formation must involve the formation of a new ring, or a new ethylenic link. With three oxide rings postulated for limonin, the carbonyl, hydroxyl, lactone and carboxyl groups account for all nine oxygen atoms of limonic acid; hence, if a new ring is formed, it must be carbocyclic.

To get further evidence, the hypiodite oxidation of tetrahydrolimonin and hexahydrolimoninic acid, and the hydrogenation of methyl limonilate were investigated.

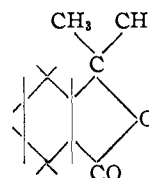
These substances were oxidized under the conditions described for limonin, but the products were more difficult to handle, presumably because the starting materials were mixtures of epimers. Tetrahydrolimonilic acid, in contrast to limonic acid, did not take up hydrogen in the presence of an active palladium catalyst, gave no color with tetranitromethane, and after the opening of the remaining lactone ring, did not readily reduce alkaline permanganate. On hydrogenation over palladium-on-charcoal in acetic acid solution, methyl limonilate yielded a mixture of a neutral substance whose analysis indicated it to be a methyl tetrahydrolimonilate, and an acid substance, the monomethyl ester of a dicarboxylic acid $C_{26}H_{36}O_9$.

Since no new ethylenic link appears to have arisen, one is forced to the conclusion that the formation of limonic acid involves the creation of a new carbocyclic ring. If one assumes that the new ring is formed by condensation between a carbon alpha to the carbonyl, and the carbon alpha to the free carboxyl of limonic acid, the added substitution about the carbonyl group might explain the shift in the position of the carbonyl band toward the visible, while the added substitution and possible inversion at the carbon alpha to the carboxyl could explain the failure to cyclize. However, the author must confess that he knows of no analogy to this postulated reaction. Obacunoic acid¹⁵ does not behave similarly.

Hexahydrolimonilic acid underwent oxidation to give a dicarboxylic acid, $C_{26}H_{36}O_9$. Hence, of the two lactone rings of limonin, one is opened by hydrogenation, and the other by limonic acid formation.

Since hexahydrolimoninic acid yields acetone on fusion with potassium hydroxide (Table I), the oxygen atom lost by hydrogenolysis is not the one eliminated as acetone, unless more than one

such atom is thus eliminated. The formation of acetone is regarded as indicative of a potential α,α -dimethyl phthalid system. Accordingly, the lactone ring opened by hypiodite oxidation may be presumed to have the carbon skeleton



We confirmed the observation of Geissman and Tulagin that limonic acid cannot be readily acetylated, which would be in keeping with the hydroxyl group being tertiary.

By treating limonin with hydriodic acid, these workers isolated two neutral substances, citrolin and desoxylimonin. In analogy to the reaction of picrotoxinin with hydriodic acid, they suggested that citrolin might be aromatic and that its intense absorption in the further ultraviolet might be due to a styrene-like chromophore. However, we felt that the broad band with a maximum at $334\text{ m}\mu$, together with the intense absorption beyond was more indicative of an α,β -unsaturated ketone.

We found citrolin to be readily obtainable, although we did not succeed in obtaining any desoxylimonin. We found the spectrum of citrolin to show a maximum at $213\text{ m}\mu$ (Figs. 1 and 2), reminiscent of the maxima of strophanthidin¹¹ or obacunone,¹⁴ in addition to the marked shoulder between 230 and $250\text{ m}\mu$. This was highly suggestive of two independent chromophores in the molecule, and by subtracting from the ϵ values of citrolin the values of obacunone in the range between 225 and $250\text{ m}\mu$, one obtains a smooth curve with a maximum about $243\text{ m}\mu$, ϵ 17,000, which is characteristic of an α,β -unsaturated ketone.

In fact the position of the maximum would be suggestive of the carbonyl group lying in a six-membered ring or an open chain with substituents α,β or β,β , and an exocyclic bond, or, if in a five-membered ring, then substituents α,β,β , with an exocyclic bond.^{16,17} As would be expected of unsaturated lactones, citrolin and obacunone reduce Tollens reagent, and, as a ketone, citrolin forms an oxime.

The analysis of citrolin and its oxime indicate citrolin to have the composition $C_{26}H_{28}O_6$. On hydrogenation over palladium, citrolin yielded a relatively small amount of acidic material, and a larger amount of a neutral fraction. The analysis of the acid product was in tolerable agreement with the composition $C_{26}H_{38}O_6 + \frac{1}{2} H_2O$, while the neutral product had the composition $C_{26}H_{36}O_6$. Octahydrocitrolin appeared saturated. That the carbonyl group was not reduced was shown by the absence of bands in the spectrum of octahydrocitrolin in the region of hydroxyl-stretching, and by the fact that this substance reacted with hydroxylamine and semicarbazide in the presence of pyridine, but unfortunately the products proved intractable. From the formula of citrolin and

TABLE I

YIELDS OF ACETONE ON POTASH FUSION

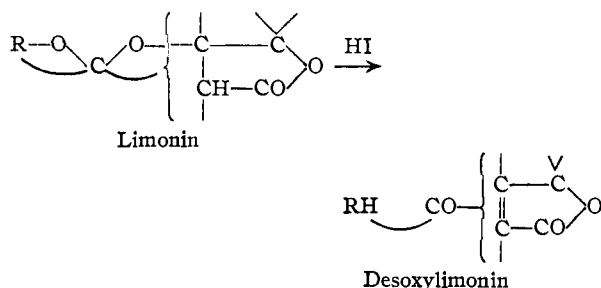
Substance	Acetone yield, mole
Limonin	0.31-0.36
Limonic acid	.16- .20
Hexahydrolimoninic acid	.12- .14
Nomilin	.12- .15

(15) O. H. Emerson, THIS JOURNAL, 73, 2621 (1951).

(16) R. B. Woodward, *ibid.*, 64, 76 (1942).(17) A. E. Gillam and T. F. West, *J. Chem. Soc.*, 486 (1942).

assuming two lactone groups, one carbonyl, one oxide ring and four ethylenic links, it follows that the substance must have three carbocyclic rings.

The most likely explanation for the formation of citrolin is that limonin has a second, potential carbonyl group in the form of a cyclic acetal grouping as postulated by Geissman and Tulagin. The system might be represented thus



One of the acetal oxygens is attached to the β -carbon of a lactone group, analogous to the attachment of the acetoxy group in nomilin, whose facile elimination results in the formation of obacunone.¹⁴ The intermediate dicarbonyl compound should have the α,β -unsaturated lactone ring, and the composition assigned to desoxylimonin. The formation of citrolin involves the further condensation of one carbonyl with a methylene group alpha to the second, forming a new carbocyclic ring, and a new ethylenic link. According to this scheme, the reduction of the R-O- group to RH requires the use of hydriodic acid, and explains why other mineral acids are ineffective in converting limonin to citrolin.

There are two reasons why one might suspect that the lactone ring which becomes unsaturated is in the potential α,α -dimethylphthalide system. First the easy liberation of acetone by the action of alkali on citrolin, observed by Geissman and Tulagin, is very suggestive. Second, the unusual strength of hexahydrolimonic acid suggests an oxygen substituent in the α -position. Were it also to have an oxygen atom attached in the β -position, one would expect the action of hydriodic acid to yield an α -keto lactone rather than an α,β -unsaturated lactone. However, the ready liberation of carbon dioxide at the melting point of hexahydrolimonic acid (170°) poses some interesting questions. Limonic acid loses only a little carbon dioxide at its melting point of 295° .

By treating citrolin with dilute alkali, we obtained a mixture of difficultly separable acids. By treating octahydrocitrolin with dilute alkali, one lactone ring was opened and on acidification octahydrocitrolin was regenerated. Under more drastic conditions, both lactone rings were apparently opened, but on acidification an oil was obtained. An attempt to regenerate octahydrocitrolin by heating this acid with acetic anhydride was unsuccessful.

Dr. C. W. Koch and Mr. V. H. Tashinian of the Chemistry Department of the University of California, Berkeley, who have been working with the Kuhn-Rohn C-methyl determination,¹⁸ very kindly

(18) These workers intend publishing shortly in *Analytical Chemistry* concerning this determination.

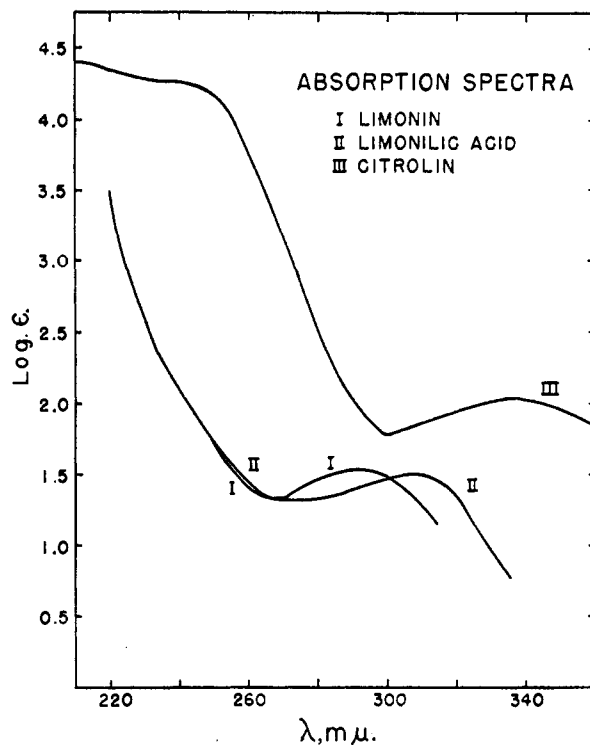


Fig. 1.—Absorption spectra: I, limonin; II, limonic acid; III, citrolin.

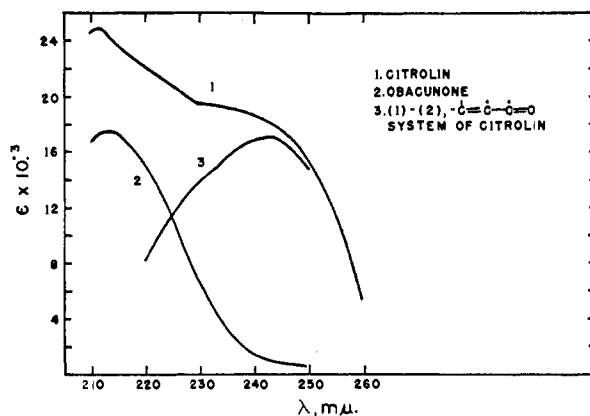


Fig. 2.—1, citrolin; 2, obacunone; 3, (1) - (2) = unsaturated ketone system of citrolin.

investigated limonin and nomilin, finding limonin to yield acetic acid equivalent to 82% of three C-methyl groups and nomilin, 85% of four. Since nomilin has an easily eliminated acetoxy group, this suggests that both compounds have the same number of methyl side-chains, and may have the same fundamental carbon skeleton. Both substances yield acetone on potash fusion (Table I) indicating at least one *gem*-dimethyl group, of which only one can be converted to acetic acid. Hence, both compounds must have at least four methyl side-chains.

Experimental

All melting points were determined with short-stemmed Anschütz thermometers.

Hydrogenation of Limonin.—Limonin (1.00 g.) was suspended in 50 ml. of acetic acid and shaken in an atmosphere of hydrogen at ordinary temperature and pressure with 1 g.

of 10% palladium-on-carbon.¹⁹ The hydrogenation was substantially complete in 8 hours, but was usually continued for 24. The catalyst was filtered off and washed with acetone, and the combined filtrates were concentrated to dryness under reduced pressure. The residue was extracted with 2% sodium bicarbonate.

The neutral tetrahydrolimonin amounted to about 100 mg., and could be recrystallized from ethanol, or more conveniently from normal butanol or methylene chloride-ethanol. The solubility characteristics were very similar to those of limonin. The crude product melted about 280°, but by recrystallization a product melting at 307–309° was obtained, though with considerable loss. A saturated solution of tetrahydrolimonin in methylene chloride gave no perceptible color with tetranitromethane, while under the same conditions limonin gives a decided yellow color. Also, in contrast to limonin, tetrahydrolimonin gives no color with concentrated sulfuric acid.

For analysis the substance was dried to constant weight at 100° at 0.1 mm.

Anal. Calcd. for $C_{26}H_{34}O_8$: C, 65.80; H, 7.23. Found: C, 65.84, 65.92; H, 7.19, 7.35.

Tetrahydrolimonin Oxime.—This was prepared as described for limonin oxime.⁵ It was recrystallized from methylene chloride-ethanol, melting at 295° after profound decomposition. For analysis it was dried to constant weight at 130° at 0.1 mm.

Anal. Calcd. for $C_{26}H_{33}NO_8$: C, 63.79; H, 7.21; N, 2.86. Found: C, 63.26, 63.27; H, 7.14, 7.45; N, 3.18.

Hexahydrolimoninic acid was obtained by acidifying the solution of the sodium bicarbonate extract of the hydrogenation product to a pH 1.0 to 1.5. The usual yield amounted to 600 to 700 mg. per gram of limonin. On recrystallization from dilute acetone it melted between 165 and 170° with the evolution of CO_2 ; further recrystallization did not significantly change its properties. The optical properties of the crystals indicated it to be a mixture which was separable with difficulty. On drying to constant weight at 110° at 0.1 mm., the crystals gave values in agreement with the monohydrate $C_{26}H_{36}O_8 + H_2O$.

Anal. Calcd. for $C_{26}H_{36}O_8$: C, 63.14; H, 7.74. Found: C, 63.27, 63.37; H, 7.70, 7.78.

The anhydrous acid was prepared by dissolving 50 mg. of the crystals in a few ml. of chlorobenzene, concentrating and adding a little iso-octane dropwise. The acid separated in an amorphous but readily filterable form, and was dried to constant weight at 100° at 0.1 mm. (approximately 8 hours).

Anal. Calcd. for $C_{26}H_{36}O_8$: C, 65.53; H, 7.62. Found: C, 65.37, 65.39; H, 7.37, 7.45.

The methyl ester was prepared by treating the acid, dissolved in a small volume of acetone or dioxane, with a slight excess of an ether solution of diazomethane. After standing a few minutes the solvents were removed and the residue recrystallized from *t*-butanol. The crude product melted at 185–190°, but after a number of recrystallizations the melting point was raised to 203–204°, but this entailed very considerable loss. Drying at 100° and 0.1 mm. to constant weight gave a product of the composition $C_{27}H_{38}O_8 + \frac{1}{2}H_2O$.

Anal. Calcd. for $C_{27}H_{38}O_8 + \frac{1}{2}H_2O$: C, 64.91; H, 7.87. Found: C, 65.10; H, 7.80.

The anhydrous ester was obtained by drying the substance to constant weight at 140° at 0.1 mm. (about 6 hours).

Anal. Calcd. for $C_{27}H_{38}O_8$: C, 66.10; H, 7.81. Found: C, 65.98, 66.01; H, 7.83, 7.64.

Limonic Acid.—Limonin (505 mg.) was hydrolyzed by refluxing 20 minutes with 150 mg. of KOH in 20 ml. of methanol. The methanol was removed under reduced pressure, and the residue was taken up in 10 ml. of water, and treated with 300 mg. of iodine dissolved with 300 mg. of KI in 10 ml. of water. Sodium hydroxide (2 *N*) was added dropwise until the solution was yellow. After standing about 15 minutes, a small amount of sodium sulfite was added, and the solution strongly acidified. After standing a couple of hours, the product was filtered off and recrystallized from isopropyl alcohol after which it melted

at 296–298° with decomposition and the evolution of carbon dioxide. The product was identical with limonic acid prepared according to Geissman and Tulagin⁴ in melting point and crystallographic properties, and both preparations yielded identical methyl esters. By working up the mother liquors, a yield of 515 mg. was obtained (98%).

For analysis the product was dried to constant weight at 100° at 0.1 mm.

Anal. Calcd. for $C_{26}H_{30}O_8$: C, 64.19; H, 6.21. Found: C, 64.06,²⁰ 64.19,²⁰ 64.23,²¹ 64.38²¹; H, 6.32,²⁰ 6.27,²⁰ 6.24,²¹ 6.17.²¹

Methyl Limonilate.—Limonic acid (105 mg.) was dissolved in 3 ml. of dioxane and treated with a slight excess of an ethereal solution of diazomethane. After standing a few minutes the solvents were removed *in vacuo* and the residue recrystallized from methanol, separating in stout prisms melting at 232–233°. For analysis the substance was dried to constant weight at 100° at 0.1 mm.

Anal. Calcd. for $C_{27}H_{32}O_8$: C, 64.78; H, 6.45. Found: C, 64.59, 64.59; H, 6.68, 6.40.

The active hydrogen was estimated by dissolving the substance (*ca.* 20 mg.) in 1 ml. of anhydrous xylene, and treating it with 1 ml. of *ca.* 1 *N* solution of methylmagnesium iodide in dibutyl ether, at room temperature. Since limonin gives variable amounts of methane when heated with the Grignard, but not at room temperature,² the solution was not heated. Found 0.71, 0.75 mole of active hydrogen calculated on the basis of a molecular weight of 500.

Methyl Limonilate Oxime.—Methyl limonilate (115 mg.) was oximated as previously described.⁵ The solvents were then removed *in vacuo*, and the residue washed with water and recrystallized from dilute methanol, separating as fine needles melting at 183–186° with decomposition. For analysis the substance was dried to constant weight at 100° at 0.1 mm.

Anal. Calcd. for $C_{27}H_{33}NO_8$: C, 62.90; H, 6.46; N, 2.72. Found: C, 63.17; H, 6.66; N, 2.90.

Hypiodite Oxidation of Tetrahydrolimonin.—Tetrahydrolimonin (285 mg.) was refluxed with 10 ml. of 0.2 *N* methanolic potassium hydroxide for 1 hour. The solvent was then removed *in vacuo*, and the residue taken up in 5 ml. of water, and treated with 250 mg. of iodine dissolved with 250 mg. of potassium iodide in 5 ml. of water. Sodium hydroxide solution (2 *N*) was added dropwise until the solution was yellow, with only a tinge of brown. After standing 15 minutes, a small amount of sodium sulfite was added, and the solution strongly acidified. After standing an hour, the product was filtered off, dissolved in sodium carbonate, and reprecipitated, then recrystallized from dilute acetone, separating as fine needles which sintered slightly at 180°, and melted with decomposition at 275°. For analysis the substance was dried to constant weight at 100° at 0.1 mm.

Anal. Calcd. for $C_{26}H_{34}O_8$: C, 63.66; H, 6.99. Found: C, 63.58, 63.65; H, 7.05, 7.02.

Attempted Hydrogenation of Tetrahydrolimonic Acid.—This was carried out in an apparatus copied after one used by Dr. J. H. C. Smith of the Carnegie Institute, whose basic features were described,²² except that he had adopted a device similar to one later reported²³ for introducing the sample into the mixture of solvent and catalyst saturated with hydrogen. A 10% palladium on charcoal catalyst (36 mg.) of a lot which had proven active in the hydrogenation of limonin was suspended in 5 ml. of acetic acid and shaken with hydrogen until saturated, then 28.2 mg. of tetrahydrolimonic acid was dropped into the mixture. There was no immediate uptake of hydrogen, but on shaking 24 hours, 0.28 ml. of hydrogen (0.2 mole) had disappeared. On a blank run with the same amount of catalyst, 0.25 ml. disappeared on shaking 24 hours, so it was felt that this loss represented hydrogen which had leaked through stopcocks and ground glass joints in the apparatus. The tetrahydrolimonic acid was recovered unchanged.

Methylation of Tetrahydrolimonic Acid.—Tetrahydrolimonic acid was methylated with diazomethane as previously described. After removing the solvents, the ester

(20) Analyses by Miss Rosie Jang, to whom the author wishes to express his sincerest thanks.

(21) Analyses by the author.

(22) J. H. C. Smith, *J. Biol. Chem.*, **96**, 35 (1932).

(23) H. Jackson and R. N. Jones, *J. Chem. Soc.*, 895 (1936).

(19) Ralph Moxingo, *Org. Syntheses*, **26**, 77 (1946). Catalyst D was used in all hydrogenations described in this paper.

was recrystallized from *t*-butanol, yielding a product melting at 181–184°. It apparently retained solvent very tenaciously, even at 130° at 0.1 mm.

Anal. Calcd. for $C_{27}H_{36}O_9$: C, 64.27; H, 7.19. Found: C, 63.50; H, 6.98.

Hypoidite Oxidation of Hexahydrolimoninic Acid.—Hexahydrolimoninic acid (509 mg.) was dissolved in 27 ml. of 0.1 *N* NaOH and heated 15 minutes on the steam-bath. The cooled solution was oxidized with 300 mg. of iodine dissolved with 300 mg. of potassium iodide in 5 ml. of water, as previously described. The product on acidification did not separate from aqueous solution but could be extracted by repeatedly shaking with an equal volume of ether. The ether residue dissolved readily in acetone and separated in good crystals on adding water to a moderately concentrated acetone solution. After it crystallized, it proved surprisingly sparingly soluble in 2-propanol and acetone, but was moderately soluble in hot dioxane and separated in beautiful prisms on carefully adding water to a saturated solution. It melted at 241–242.5°. For analysis it was dried to constant weight at 100° at 0.1 mm.

Anal. Calcd. for $C_{26}H_{36}O_9$: C, 63.40; H, 7.37; equiv. wt. (2 carboxyls), 246.3. Found: C, 63.22, 63.17; H, 7.56, 7.34; equiv. wt., 249 (titration with 0.1000 *N* NaOH).

Hydrogenation of Methyl Limonilate.—Methyl limonilate (939 mg.) was shaken in an atmosphere of hydrogen with an equal weight of 10% palladium-on-carbon. The hydrogenation was substantially complete in 6 hours, but the shaking was continued for 24. The product was worked up as previously described, yielding a relatively small amount of a neutral substance, and a much larger amount of an acid. The neutral material was recrystallized from dilute acetone, yielding a fraction melting at 197–199°. Like the ester previously obtained by methylating tetrahydrolimoninic acid, this product was difficult to dry, even at 110° at 0.1 mm.

Anal. Calcd. for $C_{27}H_{36}O_9$: C, 64.27; H, 7.19. Found: C, 63.81; H, 7.48.

The acid fraction, crystallized from dilute acetone, melted about 208°, but did not appear homogeneous, being undoubtedly a mixture of epimers. For analysis the substance was dried to constant weight at 117°.

Anal. Calcd. for $C_{27}H_{36}O_9$: C, 64.01; H, 7.56. Found: C, 63.81; H, 7.53.

Citrolin.—This was prepared as described by Geissman and Tulagin⁴ except that somewhat better yields appeared to be secured by carrying out the reaction in an inert atmosphere (CO₂). The substance is very sparingly soluble in 2-propanol, somewhat more soluble in ethyl and methyl alcohols, more soluble in acetone, and still more soluble in methylene chloride. Purification by solution in methylene chloride, followed by addition of alcohol appeared a little more convenient than acetone-alcohol. The substance melted at 305° *in vacuo*. For analysis it was dried to constant weight at 100° at 0.1 mm.

Anal. Calcd. for $C_{26}H_{32}O_8$: C, 71.55; H, 6.46. Calcd. for $C_{26}H_{30}O_8$: C, 71.22; H, 6.89. Found: C, 71.41,²⁴ 71.60,²⁴ 71.44,²⁵ 71.52²⁵; H, 6.40,²⁴ 6.50,²⁴ 6.53,²⁵ 6.41.²⁵

Oxime.—Citrolin (107 mg.) was oximated as previously described.⁵ The product was recrystallized from 2-pro-

panol. The substance had no definite melting point, as it decomposed profoundly on heating. For analysis the substance was dried to constant weight at 140°, at 0.1 mm.

Anal. Calcd. for $C_{26}H_{28}NO_8$: C, 69.17; H, 6.49; N, 3.10. Found: C, 69.12, 69.14; H, 6.33, 6.40; N, 3.26.

Hydrogenation of Citrolin.—Citrolin (380 mg.) was shaken with an equal weight of 10% palladium on charcoal in 40 ml. of acetic acid for 24 hours. After the removal of the catalyst and solvent, the residue was extracted with 2% sodium carbonate which removed a small acid fraction which was recrystallized from 2-propanol. The substance had no definite melting point. For analysis the substance was dried to constant weight at 100° at 0.1 mm.

Anal. Calcd. for $C_{26}H_{38}O_8 + \frac{1}{2}H_2O$: C, 68.55; H, 8.63. Found: C, 67.96; H, 8.77.

Lack of material prevented further investigation.

The neutral fraction (octahydrocitrolin) was recrystallized from ethanol, melting at 272–275°. For analysis it was dried to constant weight at 100° at 0.1 mm.

Anal. Calcd. for $C_{26}H_{36}O_8$: C, 70.24; H, 8.16. Found: C, 70.34, 70.19; H, 8.36, 8.14.

Hydrolysis of Octahydrocitrolin.—Octahydrocitrolin (42.0 mg.) was refluxed for half an hour with 5.00 ml. of 0.1 *N* methanolic potassium hydroxide, then diluted with an equal volume of water and back-titrated with 0.1 *N* hydrochloric acid. Equivalent weight, calculated for $C_{26}H_{36}O_8$, one lactone group opening, 444; found 480. A repetition with 32 mg. treated likewise gave equiv. wt. 470.

Octahydrocitrolin (51.0 mg.) was refluxed with 0.448 ml. of 1.230 *N* methanolic potassium hydroxide for 1 hour, then diluted with water and back-titrated with 0.1 *N* HCl. Equivalent weight found, 239.

Measurement of Acetone Formation by Potash Fusion.—The substance (*ca.* 50 mg.) was placed in a 10-ml. distilling flask with 2 ml. of 50% aqueous potassium hydroxide, and heated in a Wood's metal-bath in the course of 30 minutes to 360°. The distillate was collected in a chilled receiver and, toward the end, a very slow stream of air was passed through. The distillate was diluted with 5 ml. of acetone-free methanol, and carefully redistilled in a small still, the 5 ml. of methanol being collected, and the acetone estimated colorimetrically by reaction with the vanillin, according to the method of Alyea and Bäckström.²⁶

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(24) Analyses by the author.

(25) Analyses by V. H. Tashinian. Different samples were used.

(26) H. N. Alyea and H. L. J. Bäckström, *THIS JOURNAL*, **51**, 90 (1929).