Anal. Calcd. for $C_9H_{16}O_4N_2$: C, 50.0; H, 7.5; N, 12.9. Found: C, 50.2; H, 7.4; N, 12.8.

B. **By Curtius Degradation of (XII).**—The *trans* dihydrazide (XII) (745 mg.) was transformed into the *trans* diurethan (XIV) essentially according to the procedure used in the conversion of (XI) to (XIII). The resulting crude material, 340 mg. (39%), was recrystallized from a mixture of methanol and ether (2:1). The purified compound melted at 142–143° and was, by mixed melting point, identified with the material prepared according to A above.

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Studies on the Chemistry of Limonin

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Reinvestigation of the catalytic hydrogenation of limonin yielded two products, hydrolimoninic acid and tetrahydrolimonin, thus extending the earlier work of Koller and Czerny. A new degradation product, decarboxyhydrolimonin, has been isolated and characterized. The stepwise removal of one of the lactone groups of limonin has been achieved. The infrared absorption curves of limonin and two of its degradation products are presented.

Although the chemistry of limonin, one of the bitter principles of citrus plants, has been studied by numerous investigators, little information is as yet available on the chemical structure of this compound. Pertinent aspects of its chemistry have been summarized by Geissman and Tulagin[2] and by Emerson.[3] Limonin represents a neutral compound of the composition $C_{26}H_{30}O_8$, which is insoluble in sodium bicarbonate but readily dissolves in dilute sodium hydroxide solution. Acidification of such solutions results in a quantitative recovery of the bitter principle. Saponification studies indicate the presence of two lactone groups, while the formation of a crystalline monoxime[3] points to the presence of a carbonyl function. Thus, of the eight oxygen atoms of limonin, five are accounted for in the form of the ketodilactone functions, while the nature of the remaining three oxygens is still unknown. It is generally assumed that they are present as ether linkages.

The present investigation was undertaken to: (1) re-examine the behavior of limonin toward catalytic hydrogenation; (2) further characterize the lactone functions, and (3) obtain some insight into its basic ring skeleton.

Koller and Czerny[4] reduced limonin over a palladium-on-charcoal catalyst in absolute ethanol and observed a slow uptake of three moles of hydrogen per mole of bitter principle. The resulting amorphous reduction product readily crystallized when brought into contact with water and was shown to represent a monocarboxylic acid. The formation of this "hexahydrolimoninic" acid was postulated as involving the addition of three molecules of hydrogen and one molecule of water to the bitter principle. The acid decomposed at 175–178° and reacted with diazomethane to form an amorphous monomethyl ester. In addition to hexahydrolimoninic acid, a small amount of a neutral hydrogenation product, tetrahydrolimonin, was isolated. Similar results were obtained by Feist and Schulte-Overberg.[5] Schechter and Haller[6] also studied the hydrogenation of limonin; however, their results did not substantiate those of the previous workers. They observed a slow hydrogen uptake with the formation of a mixture of products which were not characterized. This situation prompted us to reinvestigate the hydrogenation of limonin.

Highly purified limonin, which was recrystallized from glacial acetic acid and consequently contained acetic acid of crystallization, served as the starting material. Hydrogenation of this material, over a palladium-on-charcoal catalyst with ethanol as the solvent, resulted in a rapid uptake of three moles of hydrogen per mole of bitter principle. Several hydrogenations of limonin were carried out with essentially similar results. Evaporation of the solvent gave an amorphous material which crystallized on the addition of water. Recrystallization from dilute ethanol afforded a monocarboxylic acid melting with decomposition at 163–165°. Neutral equivalent determinations and elementary analyses pointed to a formula of $C_{26}H_{36-38}O_9$ for this compound. A crystalline substance with properties identical to those reported for tetrahydrolimonin was isolated from the neutral hydrogenation products.

The properties of our acidic hydrogenation product are similar to those reported for hexahydrolimoninic acid[4]; however, certain differences are noteworthy. The decomposition point of hexahydrolimoninic acid is given as 175–178°; in contrast, our acid decomposed consistently at 163–165°. Also, the analytical figures and neutral equivalents obtained in our laboratory differ somewhat from those previously reported (see Experimental). Although no explanation can be offered for these differences, it seems reasonable to assume the identity of the products; their similar behavior on heating, as discussed below, also supports this conclusion. Limonin absorbs three moles of hydrogen under the experimental conditions employed; however, the acidic reduction product was obtained in only 40–50% yields. Consequently, we cannot exclude the possibility of competing reactions simulating an over-all uptake of three moles of hydrogen and leading to more highly hydrogenated amor-

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(1) We wish to express our gratitude to Dr. W. E. Baier, of the California Fruit Growers Exchange, for generous gifts of Valencia orange seeds, and to Dr. F. A. Miller, of the Mellon Institute of Industrial Research, for the infrared absorption spectra.

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

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

(4) G. Koller and H. Czerny, *Monatsh.*, **67**, 267 (1936); *ibid.*, **70**, 26 (1937).

(5) K. Feist and L. Schulte-Overberg, *Ber.*, **69**, 1322 (1936).

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

phous products. The analytical figures obtained on several samples of the acidic hydrogenation product would agree with a tetra- or a hexahydro derivative. Therefore, hydrolimoninic acid represents a preferable name for this compound.

In addition to an increased hydrogen content, hydrolimoninic acid also differs from limonin by one molecule of water. It remains to be determined how this water is incorporated into the molecule, *i.e.*, as water of crystallization or by lactone ring opening with the formation of a hydroxycarboxylic acid. Obviously the formation of hydrolimoninic acid from the neutral limonin molecule must involve the opening of one of the lactone rings. The infrared absorption spectrum of hydrolimoninic acid (Fig. 1) exhibits an absorption peak at 3650 cm.$^{-1}$, which may be due to a hydroxy group or to water of hydration.

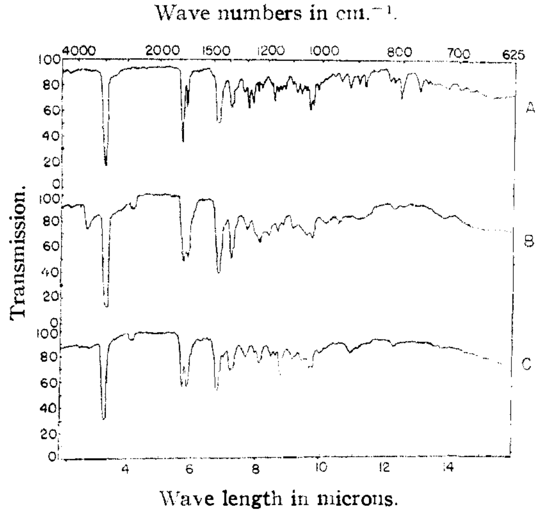

Fig. 1.—Infrared absorption spectra of: A, limonin; B, hydrolimoninic acid; C, decarboxyhydrolimonin. The measurements were carried out in Nujol mull with a Baird spectrophotometer with rock-salt prism.

Koller and Czerny[4] heated hexahydrolimoninic acid at 180° and observed that the compound decomposed with the liberation of approximately one mole of carbon dioxide and two moles of water. They failed to investigate the nature of the resulting decarboxylation products. In order to further substantiate the identity of our acidic hydrogenation product with hexahydrolimoninic acid, its behavior on heating was investigated. Heating the compound at 240° led to a rapid evolution of carbon dioxide and water. Quantitative determination of the liberated carbon dioxide demonstrated that 0.7 mole of carbon dioxide was given off per mole of hydrogenation product. A new crystalline compound, which we call decarboxyhydrolimonin, was obtained from the glassy reaction products. The substance is neutral, melts at 215–219°, and may be sublimed *in vacuo*. The analytical figures point to a composition of $C_{25}H_{34-36}O_6$. The compound fails to absorb oxygen when exposed to monoperphthalic acid. Molecular weight determinations by the Rast method gave a molecular weight of 378, which is in fair agreement with the expected molecular weight of 430–432. Therefore, no deep-seated cleavage into smaller fragments had occurred during the heat treatment. The saponification equivalent of 440 demonstrated the presence of a lactone grouping in decarboxyhydrolimonin. This lactone seems to be responsible for the absorption maximum at 1760 cm.$^{-1}$ in the infrared absorption curve of decarboxyhydrolimonin (Fig. 1). The maximum at 3650 cm.$^{-1}$ present in the spectrum of hydrolimoninic acid is absent in the infrared absorption curve of decarboxyhydrolimonin, indicating the loss of a water molecule during the heat treatment.

With the exception of differences in the analytical figures of hydrolimoninic acid, our results substantiate the findings of Koller and Czerny.[4] In addition, they provide a convenient path for the elimination of one of the lactone groups of limonin. Decarboxyhydrolimonin represents a useful starting material for further investigations on the structure of this bitter principle.

The infrared absorption spectrum of limonin, which exhibits a pronounced maximum at 1760 cm.$^{-1}$ (Fig. 1) points to the presence of a γ-lactone. The presence of an α,β-unsaturated γ-lactone seems unlikely since such structures exhibit a characteristic maximum at 220 mμ,[7] which seems to be lacking in the ultraviolet absorption spectrum of limonin.[2] The exact course of the hydrogenation leading to the formation of hydrolimoninic acid is not understood and requires further study.

Attempts to transform limonin into crystalline phenylhydrazides or hydrazides by treatment with phenylhydrazine or hydrazine were unsuccessful. Limonin failed to react with phenylhydrazine under the experimental conditions employed and only amorphous materials were obtained from its reaction with hydrazine.

The formation of characteristic aromatic hydrocarbons on dehydrogenation of natural materials has frequently given valuable information about their basic carbon skeleton. Thus, limonin and hydrolimoninic acid were subjected to dehydrogenation with selenium at temperatures of 300–350°. Despite extensive fractionation studies of the resulting dehydrogenation products, no aromatic hydrocarbons could be isolated.

Experimental[8]

Isolation of Limonin. a. From Navel Orange Peel Meal.—The isolation of the bitter principle from this source was carried out essentially according to the procedure of Geissman and Tulagin.[2] The crude limonin (yield 0.4 g. per lb. of meal) was purified by recrystallization from glacial acetic acid and was thus obtained as limonin acetic acid solvate.[9]

b. From Valencia Orange Seeds.—The procedure of Emerson[3] was used to isolate the bitter principle from this raw material. It was purified by recrystallization from a mixture of methylene chloride and ethanol and finally from glacial acetic acid; yield 1 g. of pure limonin per lb. of seeds; m.p. 298° with decomposition. The properties of the limonin acetic acid solvate obtained from both sources agreed with those previously reported in the literature. The analytical samples were dried at 100°, 0.8 mm., for four hours.

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[7] L. Ruzicka, P. A. Plattner and A. Fürst, *Helv. Chim. Acta*, **25**, 79 (1942).

[8] The microanalyses were performed in our Microanalytical Laboratories by Mr. George L. Stragand; the melting points were determined with short-stem Anschütz thermometers and are uncorrected.

[9] F. T. Jones and K. J. Palmer, THIS JOURNAL, **71**, 1935 (1949).

Anal. Calcd. for $C_{26}H_{30}O_8(C_2H_4O_2)$: C, 63.4; H, 6.5. Found: C, 63.3; H, 6.6. *Rotation.* Found: $[\alpha]^{26}D$ −122° (in acetone). Reported[2,3,6]: $[\alpha]^{25}D$ −123°.

Hydrogenation of Limonin.—Eight separate hydrogenations of limonin were carried out during this investigation. One typical experiment was carried out as follows: A suspension of limonin acetic acid solvate (4.0 g.) and 5% palladium-on-charcoal catalyst[10] (4.0 g.) in absolute ethanol (150 ml.) was shaken in the presence of hydrogen. Hydrogen was rapidly absorbed and the limonin gradually dissolved. After 360 minutes the hydrogenation came to an end when 556 ml. of hydrogen, standard conditions, had been absorbed—calculated on the basis of three moles, 570 ml. at standard conditions. The reaction mixture was filtered through Hyflo Supercel and the palladium–charcoal residue re-extracted twice with hot, absolute ethanol. The combined filtrates and washings were concentrated to dryness *in vacuo* and the amorphous residue suspended in water (10 ml.). The suspension was warmed on a steam-bath and 95% ethanol was added until a clear solution resulted; this was filtered and placed in a refrigerator. The crystals of hydrolimoninic acid were collected, washed with cold water and dried *in vacuo* over phosphorus pentoxide. The acid (2.06 g.) (49%) melted with decomposition at 163–165°.

The analytical samples were dried at 100°, 0.8 mm., for three hours over phosphorus pentoxide.

Anal. Calcd. for $C_{26}H_{36}O_9$:	C, 63.4;	H, 7.4;	neut. equiv., 492;
for $C_{26}H_{38}O_9$:	C, 63.2;	H, 7.7;	neut. equiv., 494.
Found (this investigation)	C, 63.6;	H, 7.3	neut. equiv.,[11] 488
	63.1	6.9	507
	63.1	7.2	506
	63.1	7.4	508
	63.7	7.6	
	63.5	7.3	
Average	C, 63.3;	H, 7.3.	502
Found (previous investigations,[4] average)	C, 62.7;	H, 8.1;	neut. equiv.,[12] 463.

Tetrahydrolimonin.—The mother liquors from the recrystallization of hydrolimoninic acid were evaporated and the recovered material dissolved in 10% sodium bicarbonate (60 ml.). The resulting solution was vigorously shaken with three 40-ml. portions of chloroform and the combined chloroform extracts dried over sodium sulfate. Evaporation of the chloroform left a solid, which was recrystallized from dilute ethanol. The resulting crystals (88 mg.) melted with decomposition at 279–280° and their properties agreed with the tetrahydrolimonin[4] previously reported with melting point at 282°.

Anal. Calcd. for $C_{26}H_{34}O_8$: C, 65.8; H, 7.2. Found (this investigation): C, 65.9; H, 6.9. Found (previous investigation[4]): C, 65.8; H, 7.4.

Decarboxylation of Hydrolimoninic Acid.—Hydrolimoninic acid (2 g.) was placed in a long-neck, 50-ml., round-bottomed flask fitted with a gas inlet tube and connected to an absorption vessel containing standardized barium hydroxide solution. After a 15-minute flushing of the system with a slow stream of nitrogen, the flask containing the substance was lowered into a Woods metal-bath previously heated at 240°. The acid immediately melted with the evolution of water and carbon dioxide. The decarboxylation ceased after 15 minutes; the carbon dioxide was swept into the barium hydroxide solution by nitrogen and determined by titration with hydrochloric acid using thymol blue as the indicator. An amount of carbon dioxide representing 0.7 mole per mole of hydrolimoninic acid was obtained. The residue in the reaction flask cooled to a brown glassy substance, which was dissolved in hot ethanol. The solution was treated with Norite and filtered. The clear filtrate on standing overnight deposited crystals, which were collected, recrystallized from ethanol and dried. Square crystals (148 mg.) were obtained which melted at 215–219°. Several decarboxylations were carried out with similar results.

The analytical samples were dried over phosphorus pentoxide for three hours at 100°, 0.8 mm.

Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.8; H, 7.9; sapon. equiv., 430; mol. wt., 430; calcd. for $C_{25}H_{36}O_6$: C, 69.4; H, 8.4; sapon. equiv., 432; mol. wt., 432. Found: C, 69.2, 69.4, 69.4; H, 8.0, 7.8, 7.8; sapon. equiv.,[13] 460, 426, 436; mol. wt.,[14] 377, 378.

Monoperphthalic Acid Oxidations.—Decarboxyhydrolimonin failed to absorb oxygen upon exposure for 48 hours to monoperphthalic acid in dioxane solution.[15] Methyl oleate under identical conditions absorbed one equivalent of oxygen.

Reaction of Limonin with Phenylhydrazine and Hydrazine.—The reaction between the bitter principle and phenylhydrazine was carried out essentially according to Huntress and Mulliken.[16] A typical experiment was carried out as follows: A solution of limonin (0.313 g.) and freshly distilled phenylhydrazine (0.266 g.) in dioxane (20 ml.) was placed in a small flask and refluxed for 18 hours. The solvent was removed *in vacuo* leaving a viscous oil which was stirred with ether to give a powder (0.282 g.). This material was recrystallized and identified as unchanged limonin.

Treatment of the bitter principle with hydrazine under a number of experimental conditions afforded gummy materials which could not be crystallized.

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[10] Obtained from the American Platinum Works, Newark, N. J.

[11] Determined potentiometrically in 50% ethanol with a Beckman line-operated *p*H meter.

[12] Determined in aqueous ethanol by the use of phenolphthalein.

[13] The saponification equivalents were determined according to the method of F. Schneider, "Qualitative Organic Microanalysis," John Wiley and Sons, Inc., New York, N. Y., 1946, p. 161.

[14] Determined in camphor according to the Rast method.

[15] H. Böhme, "Organic Syntheses," Vol. 20, John Wiley and Sons, Inc., New York, N. Y., 1940, p. 70.

[16] E. H. Huntress and S. P. Mulliken, "Identification of Pure Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1941, p. 352.