Solvents other than 95% alcohol were tried, but all these—glacial acetic acid, or a mixture of dioxane and water to which three equivalents of concentrated hydrochloric acid per mole of compound reduced had been added, or absolute alcohol containing three equivalents of anhydrous hydrogen chloride as reduction media—gave considerably lower yields of amine salt. The yield was much lower when the amount of isonitroso compound reduced per given volume of solvent was greater than 35 g./450 cc., but a decrease in the amount reduced per given volume of solvent failed to increase the yield.

Methyl Propiophenone-*o*-carboxylate (V, R = CH_3).—*o*-Carboxypropiophenone, which was prepared by the methods used to prepare *o*-carboxybutyrophenone, was esterified in 87% yield; b. p. 137 (5 mm.) to 132° (4 mm.), n^{20}_{D} 1.5260–1.5290 (lit.,[7] b. p. 157–158° (19 mm.), $n^{16.4}_{\text{D}}$ 1.5240).

Methyl α-Isonitrosopropiophenone-*o*-carboxylate (VI, R = CH_3).—This compound was recrystallized from methanol, m. p. 166–169° (Fisher block).

Anal. Calcd. for $C_{11}H_{11}NO_4$: C, 59.72; H, 5.01; N, 6.33. Found: C, 59.84, 59.97; H, 4.93, 4.65; N, 6.33, 6.29.

1-Amino-1-phthalidylethane Hydrochloride, Isomer A (VIII, R = CH_3).—This compound was obtained in 64.9–74.5% yield and after recrystallization melted in the range 286–295° (lit.,[1] 292–296° dec.).

1-*p*-Nitrobenzoylamino-1-phthalidylethane (A) melted 188–189.5° after crystallization from alcohol (lit.[1] 188–189°).

Anal. Calcd. for $C_{17}H_{14}O_5N_2$: C, 62.57; H, 4.29; N, 8.58. Found: C, 62.47, 62.58; H, 4.29, 4.35; N, 8.54, 8.59.

Summary

1. A new synthesis of 1-amino-1-phthalidylalkanes has been reported.

2. This synthesis resulted in only one of the possible racemic forms of 1-amino-1-phthalidylethane and -propane.

3. In the preparation of the intermediate, methyl butyrophenone-*o*-carboxylate, the product underwent partial decomposition to propylidenephthalide unless carefully freed of acid before distillation.

4. Observations have been reported on the relationship between normal and pseudo-esters of butyrophenone-*o*-carboxylic acid in regard to their ultraviolet absorption spectra and their ability to undergo the isonitroso reaction.

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[7] Beilstein X-S, p. 334.

[CONTRIBUTION NO. 109 FROM ENZYME RESEARCH LABORATORY, BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY, AGRICULTURAL RESEARCH ADMINISTRATION, USDA]

The Bitter Principles of Citrus Fruit. I. Isolation of Nomilin, a New Bitter Principle from the Seeds of Oranges and Lemons

BY OLIVER H. EMERSON

Limonin is a well recognized bitter principle in citrus plants that has been regarded as responsible for the bitter taste which sometimes appears in the juice of Navel oranges on standing or heating. Evidence which favors that view is presented here and points to a reaction whereby limonin may be formed from a precursor in orange juice. In addition a new bitter principle, "nomilin," has been isolated from the seeds of oranges and lemons. It appears closely related to limonin, and because of the similarity in properties it may also contribute to the bitter taste.

Limonin was first isolated by Bernay in 1841 and has since been obtained from a series of citrus and related *Rutaceae*,[1,2,3] having been found in the fleshy part of the fruit, the seeds, the bark and the roots. The chemical nature of the bitter principle has recently been discussed by Geissman and Tulagin.[4] It is well established that the substance is a dilactone of the formula $C_{26}H_{30}O_8$. It has no reactive hydroxyl groups, giving no methane with cold methylmagnesium iodide,[5] although Koller and Czerny reported it to give variable amounts when heated, which suggested to them the presence of a carbonyl group capable of enolization, although no carbonyl derivatives have been reported. No methoxyl or methylene dioxy groups have been detected, and the remaining three oxygen atoms are presumably present as cyclic ethers. Drastic treatment with potassium hydroxide yields 0.88 mole of acetone.[4]

The question of a carbonyl group, as postulated by Koller and Czerny, is obviously of importance to the problem of limonin. The absorption spectrum of limonin as measured by Geissman and Tulagin and in this Laboratory (Fig. 1) shows a weak band at about 290 mμ, $\log E = 1.4$, which is suggestive of an isolated carbonyl group, though such a weak band could easily result from traces of an impurity. However, we have succeeded in preparing a beautifully crystalline oxime by treating limonin with hydroxylamine in the presence of pyridine, which will often permit the oximation of carbonyl groups that fail to react with hydroxylamine acetate.[6]

Isolimonin.—Besides limonin, Koller and Czerny[5] reported the isolation from orange seeds of an isomer of limonin melting at 258–259° which they called isolimonin. It crystallized from alcohol in thick "warty" crystals in contrast to the thin six-sided plates of limonin and differed from

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[1] Schechter and Haller, THIS JOURNAL, **62**, 1307 (1940). This paper summarizes excellently the earlier literature.

[2] Mookerjee, *J. Indian Chem. Soc.*, **17**, 593 (1940).

[3] Seshadri and Veeraragavia, *Proc. Indian Acad. Sci.*, **11A**, 505 (1940).

[4] Geissman and Tulagin, *J. Org. Chem.*, **11**, 760 (1946).

[5] Koller and Czerny, *Monatsh.*, **67**, 248 (1936).

[6] Bachmann and Boatner, THIS JOURNAL, **58**, 2097 (1936).

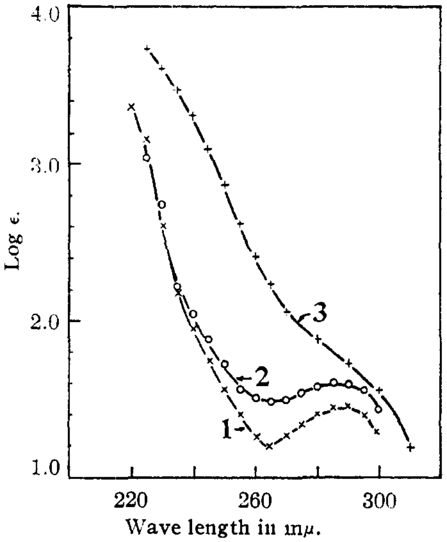

Fig. 1.—Absorption spectra in alcohol: (1) limonin; (2) nomilin; (3) nomilic acid.

that substance in being more soluble in alcohol, less soluble in benzene, in readily forming a diacetyl derivative and in giving two moles of methane with methylmagnesium iodide.

A bitter principle from very immature Navel oranges was investigated by Higby[7] who isolated it as shiny plates melting at 262–264°, $[\alpha]^{22}D$ $-111.5°$. He thought the substance to be isolimonin in view of the similarity in rotation and melting point. From the seeds of Valencia oranges he obtained limonin melting at 292°. Later he reported that the usual bitter constituent, at least in more mature fruit, is limonin,[8] a view in which Geissman and Tulagin concur. While other references to isolimonin occur in the literature,[2,3] it must be admitted that its characterization leaves much to be desired.

The isolation of the bitter principle from Navel orange juice as described by Higby[7] was repeated except that the final purification was done by solution in methylene chloride followed by the addition of alcohol, as described by Feist and Overberg.[9] Limonin was obtained agreeing in melting point[1,4,9] and optical properties[10] with the findings of other workers[1] and with preparations made in this Laboratory from the seeds of Valencia oranges and of lemons. From methylene choride–alcohol limonin separates in part as the characteristic unsolvated plates and in part as a loose solvate with methylene chloride crystallizing in needles, as described by Feist and Overberg for "citrolimonin" from lemon seeds.[11]

In an attempt to obtain isolimonin, ground and defatted Valencia orange seeds were extracted with acetone instead of the previously used alcohol[5] in the belief that the greater solubility of the bitter substances in the former solvent would make it more suitable. However, it was found after fairly exhaustive extraction that the seed meal was still intensely bitter. To water this extracted meal imparted a very bitter taste which disappeared on standing. The bitter substance was not extracted either by absolute or 95% ethanol but was extracted by 50% ethanol, or more conveniently by 1-butanol saturated with water. However, the chief bitter substance isolated was limonin. Thinking that this substance might be present as an unstable derivative soluble in water-containing organic solvents, we tried extraction under a variety of conditions in the hope of securing such a derivative but without success. It was noticed that the solvents which extracted limonin also removed considerable amounts of gummy material which may have interfered with the extraction of limonin by the dry solvents and permitted the formation of a transient, bitter colloidal solution in water. Only about one-third of the total limonin obtained was found in the acetone extract.

Nomilin.—From the mother liquors of limonin from both orange seeds and lemon seeds a second bitter principle was obtained. It was easily separated from limonin since it remained in solution in ethanol containing methylene chloride and also dissolved in hot 2-propanol, in which limonin is very sparingly soluble. It was further purified by recrystallization from methanol, from which it separated in beautiful needles melting at 279°, though unfortunately the melting point is somewhat dependent on the rate of heating.

The substance is notable for its tendency to form solvates with many solvents, which causes the appearance of crystals from different solvents to differ markedly. Several of the solvates required heating at 140° at 1 mm. for their decomposition.

In order to get reliable analytical data samples of the substance were recrystallized from 2-propanol, 1-butanol, ethyl acetate and methylene chloride–petroleum ether. Each sample was dried to constant weight at 140° at 1 mm. and analyzed. The agreement was satisfactory and in conjunction with the accurate Signer method[13,14] indicated the molecular formula to be $C_{28}H_{34}O_9$.

Since the substance is not an isomer of limonin though closely associated with it and presumably related to it chemically the name **nomilin** is proposed. Nomilin may be quite an important constituent of the total bitter material of orange seeds. Thus one batch of 17 kilos yielded 24 g. of limonin and 14 g. of nomilin.

It is conceivable that the isolimonin of Koller and Czerny was impure nomilin, but the Austrian workers reported their substance to give two moles

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(7) Higby, This Journal, **60**, 3013 (1938).

(8) Higby, *California Citrograph*, **26**, 360 (1941).

(9) Feist and Overberg, *Ber.*, **69**, 1322 (1936).

(10) The optical properties of many of the crystalline preparations obtained were examined by Dr. Francis T. Jones, for whose kind cooperation I wish to express my sincerest thanks.

(11) The identity of "citrolimonin" with limonin, assumed by other workers,[1,9,12] is thus further substantiated.

(12) Koller and Czerny, *Monatsh.*, **70**, 26 (1937).

(13) Signer, *Ann.*, **478**, 246 (1930).

(14) Clark, *Ind. Eng. Chem., Anal. Ed.*, **13**, 820 (1941).

of methane with methylmagnesium iodide and to yield readily a diacetyl derivative, whereas nomilin has only one active hydrogen and is acetylated with difficulty if at all by refluxing for several hours with acetic anhydride and sodium acetate or with acetic anhydride and pyridine. In both cases the bulk of the nomilin was recovered unchanged, though some material was obtained whose high carbon content indicated the elimination of the hydroxyl rather than its acetylation.

The absorption spectrum of nomilin is shown in Fig. 1.[15] The similarity to that of limonin is striking. Like limonin, nomilin did not react with carbonyl reagents except under the powerful activating influence of pyridine. In contrast to the beautifully crystalline oxime of limonin, the oxime, semicarbazone and 4-phenylsemicarbazone of nomilin were amorphous products whose melting points, owing to decomposition, were indefinite.

Like limonin, nomilin is a dilactone and the lactone groups are readily opened by dilute alkali. In the case of nomilin the reaction was more complicated for acetic acid was split out, and on acidification there was obtained a lactone-acid for which the name *nomilic acid* is proposed. The analysis and titration of the free carboxyl group indicated the formula $C_{26}H_{32}O_8$. The spectrum is shown in Fig. 1. The intensification of the absorption and the shift toward the visible suggests a greater degree of conjugation in nomilic acid than exists in limonin or nomilin.

On fusion with potassium hydroxide at 300–340° nomilin, in contrast to limonin, did not give significant amounts of acetone.

Other Bitter Principles.—A third bitter substance (substance X) was obtained from the crude alcoholic mother liquors of orange seeds. It differed from limonin and nomilin by its very sparing solubility in methylene chloride and acetone. The solubility of the substance was greatest in ethyl and methyl alcohols among the common solvents tried, but even in these the product was only sparingly soluble and had a low temperature coefficient of solubility. The best preparation separated from methanol on concentration or on dilution with water in the form of delicate hair-like needles melting with profound decomposition at 315°. The Molisch test was negative. The analysis agreed with the formula $(C_9H_{12}O_4)_n$, but in view of the strong retention of methanol by nomilin encountered later this may be in error. The spectrum was similar to that of limonin but more generalized, with no suggestion of any definite bands. Purification was difficult because of the presence of amorphous material of similar solubility. Since it was a very minor constituent of orange seeds it was not further investigated. It was not observed in lemon seeds.

Development of the Bitter Substances in Navel Orange Juice.—Navel oranges are an important crop in California and the tendency of the juice to turn bitter is of serious concern. The problem was studied by Higby,[7] who concluded that in the intact orange limonin exists in the albedo and pulp in the form of a water-soluble, non-bitter precursor which might be the salt of the diacid derived by opening both lactone rings, or a glycosidic derivative of it.

The formation of the bitter substance was observed by extracting Navel juice with benzene as soon as possible after the juice was expressed, then again after four hours and again after standing overnight. The juice after the removal of benzene was not perceptibly bitter and the residues from the first and last extractions were only slightly bitter, the bulk of the bitter material being found in the second extract. Since limonin was the only bitter substance isolated from the benzene extracts it must be chiefly responsible for the bitter taste.[16] Also, whatever the precursor, the rate of conversion to limonin was such that in orange juice at *p*H 3.6 comparatively little was formed in the twenty minutes required for the first extraction, but the conversion was essentially complete in four hours. Potassium limonate equivalent to the amount isolated from bitter Navel juice was added to Valencia juice which does not normally turn bitter, also at *p*H 3.6, and the development of bitterness observed appeared to parallel that in Navel juice both in degree and rate.

Higby's[7] suggestion that the precursor of limonin in Navel orange juice is the diacid or a derivative of it such as the glucoside appears to be close to the truth, although conceivably the precursor might be one of the lactone acids since the related lactone acids, limonilic acid[4] and nomilic acid are not bitter. Accordingly, in view of the tendency of the lactone rings to reestablish themselves in acid solution, the precursor can be stable only in more nearly neutral tissues like the albedo, central bundle, and veins of the capillary membrane. The conversion of the precursor to limonin is a process catalyzed by hydrogen ion and by heat and is inhibited by neutralizing the juice which, however, makes it unpalatable. There is at present no evidence that enzymes are involved in the process.

Experimental

Limonin from Navel Oranges.—Immature Navel oranges (140 lb.) collected near Los Angeles about the middle of October, 1945, yielded five gallons of juice, which was strained through cheese cloth and thoroughly extracted with benzene. The pulp strained from the juice was extracted separately and the extract from it was united with that from the juice. The peel, consisting of flavedo with some albedo, was extracted with petroleum ether to remove the oil, pressed as dry as possible in a hydraulic press and then extracted with benzene. The benzene extracts were concentrated to 200 ml. and the limonin precipitated by the addition of three volumes of petroleum ether. Most of the product came from the juice and pulp, and the small amount from the peel may

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[15] I wish to express my sincerest thanks to Mr. G. F. Bailey and to Mrs. Martha Adkins for measuring the absorption spectra reported in this paper.

[16] The isolation was done before the discovery of nomilin, which could easily have escaped detection.

well have come from albedo accompanying it. The crude limonin was purified by solution in a minimum amount of methylene chloride followed by the addition of three volumes of methanol or ethanol. A few recrystallizations brought the melting point up to 298–300°.[17] In an evacuated tube the substance melted at 301.5–302.5°. Previously reported melting points for limonin are: 299–300°,[1] 302–305,[4] 304.[9] The yield was 1.58 g.

A second lot of oranges (140 lb.) collected from the same area early in November, 1945, yielded 2.5 g. of limonin.

From methylene chloride, on dilution with ethanol or methanol, limonin separated as needles accompanied by the more characteristic plates. On heating *in vacuo* the needles readily lost methylene chloride which was trapped and identified. Needles (144 mg.) picked out with forceps and heated at 80° *in vacuo* lost 18.3 mg. or 12.7% of their weight. The complex $C_{26}H_{30}O_8 \cdot CH_2Cl_2$ contained 15.4% of methylene chloride by calculation. Even on standing in air the needles lost methylene chloride and became opaque.

Anal. Material dried at 80° at 1 mm. for 1 hour. Calcd. for $C_{26}H_{30}O_8$: C, 66.35; H, 6.42. Found: C, 66.45; H, 6.49.[18]

Rotation.—Material dried as above (227.9 mg.) made up to 25 ml. in acetone in a 2-dm. tube at 22° gave a rotation of −6.35 units in a saccharimeter; $[\alpha]^{22}\text{D}$ −121°. Reported $[\alpha]^{20.5}\text{D}$ −129°,[1] $[\alpha]^{25}\text{D}$ −123°.[4]

Isolation of Limonin from Valencia Orange Seeds.—Orange seeds (22 lb.) were ground and defatted with petroleum ether and extracted with acetone. The acetone solution was concentrated to a thin sirup, diluted with ethanol and concentrated again, yielding most of the limonin. A little more was secured by the addition of water. Purification was done by crystallization from methylene chloride and alcohol as described above. The yield was 13 g.

The acetone-treated meal was then well extracted with 1-butanol saturated with water, and the extracts concentrated under reduced pressure. Some gummy water-soluble material separated first and was discarded. The bitter substances present separated in impure form on further concentration. These were filtered off. The butanol was finally concentrated to a sirup, diluted with ethanol and then with a little water, throwing out a last crop of bitter material.

The combined portions of crude bitter material were treated with acetone and a residue of some non-bitter material was removed. The acetone solution was concentrated to small volume and then diluted with ethanol. This precipitated the bitter substances still in an impure state. Purification was made by dissolving this material in methylene chloride and precipitating limonin by the addition of 3 volumes of ethanol.[19] This step was repeated until the limonin had a melting point of 298–300°. The total yield of pure liminon (after recrystallization) obtained from 22 lb. of orange seeds was 40 g., though a second batch of 37 lb., extracted directly with wet butanol, yielded only 24 g.

Isolation of Nomilin.—On concentrating the methylene chloride–alcohol mother liquors of limonin obtained on recrystallizing the bitter fractions described above there separated a mixture of a new substance and limonin. Nomilin was extracted from the mixture by boiling for a few minutes with a minimum amount of 2-propanol and filtering the hot solution. On cooling nomilin separated as stout crystals. Further purification was secured by recrystallization from methanol from which nomilin separated in slender needles. Nomilin is soluble to the extent of 1.5 to 2% in boiling methanol and in boiling 2-propanol, and somewhat more soluble in hot ethyl acetate. In other solvents its solubility is generally similar to that of limonin. The yield of nomilin from 37 lb. of orange seeds was 14 g.

On account of decomposition the melting point of nomilin is not very definite. With a block heated at the rate of 2° per minute it melted at 278–279° with evolution of gas when introduced at 273–275°. The use of a melting point tube sealed *in vacuo* did not sharpen the melting point as it did in the case of limonin.

For analysis samples were recrystallized from ethyl acetate, 2-propanol, 1-butanol and methylene chloride followed by the addition of petroleum ether. Each sample was dried to constant weight at 140° at 1 mm. The results are shown in Table I.

TABLE I

ANALYSES OF NOMILIN

Solvents used for crystallization	C	H	Molecular weight (Signer)
Butanol	65.45	6.90	
Butanol	65.46	6.75	
Isopropanol	65.57	6.81	
Isopropanol	65.26	6.77	
Methylene chloride	65.53	6.73	
Petroleum ether	65.35	6.63	
Ethyl acetate	65.30	6.74	511
Ethyl acetate			516
Average	65.42	6.67	513
Calculated for $C_{28}H_{34}O_9$	65.33	6.67	514.3

Molecular Weight.—This was done by the Signer method as described by Clark.[14] Owing to the difficulty of securing accurately calibrated Pyrex tubing for the volumetric part of the apparatus, ordinary 4.5–5.0 mm. tubing was used, and the lengths of the columns of solution were measured to an accuracy of about one-fourth mm. with a rule having a little foot to rest against the end of the tube. The corresponding volume was determined by means of mercury.

Nomilin (33.5 mg.) from ethyl acetate, dried as above in 1.075 ml. of acetone, was in equilibrium with 12.85 mg. of azobenzene in 1.158 ml., mol. wt., 511. Nomilin (33.2 mg.) in 0.855 ml. of acetone was in equilibrium with 13.81 mg. of azobenzene in 1.006 ml., mol. wt., 516.

Rotation.—Material recrystallized from 2-propanol and dried as above (298.9 mg.) made up to 25 ml. in acetone in a 2-dm. tube gave a rotation of −6.60 units in a saccharimeter at 23°.

$$[\alpha]^{23}\text{D} = -\frac{6.60 \times 0.346 \times 100}{2 \times 1.195} = -95.7°$$

Isolation of Limonin and Nomilin from Lemon Seeds.—Lemon seeds (15 lb.) were ground, defatted and extracted with 1-butanol as described for the orange seeds. Nomilin and limonin were separated and isolated as described above. The optical properties of the crystals and melting points coincided with the products from orange seeds.

Rotation of Nomilin from Lemon Seeds.—Material dried as described above, 273.8 mg., made up to 25 ml. in acetone in 2-dm. tube in a saccharimeter at 23°, rotation −6.05 units

$$[\alpha]^{23}\text{D} = -\frac{6.05 \times 0.346 \times 100}{2 \times 1.095} = -95.7°$$

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[17] All melting points were done in a copper block using short stemmed Anschütz thermometers and the substance was introduced a few degrees below the melting point.

[18] I wish to thank Dr. W. Gordon Rose for instruction and help in running microanalyses.

[19] The use of 2 volumes of 2-propanol instead of 3 volumes of ethanol was later found to be of advantage.

Reaction of Nomilin with Methylmagnesium Iodide.[20]—Nomilin (9.92 mg.) in pyridine gave 0.50 ml. of methane at 22°, 741 mm. Nomilin (11.59 mg.) in pyridine gave 0.57 ml. of methane at 22°, 741 mm. Calcd. for $C_{28}H_{34}O_9$: 1.05, 1.02 atoms of active H.

Action of Dilute Alkali on Nomilin.—Nomilin (237 mg.) was heated on the steam-bath with 40 ml. of 0.1 *N* NaOH with occasional swirling. After half an hour the solution was cooled, filtered from a small amount of undissolved material and acidified with 10 ml. of 0.5 *N* HCl. The crude nomilic acid which soon separated was purified by solution in a minimum amount of acetone followed by the addition of an equal volume of water, separating as beautiful needles, m. p. 205–206°; yield 190 mg. (88%). Further recrystallization brought the melting point up to 208–209° in an evacuated tube.

Anal.—Material dried to constant weight at 100°, 1 mm. Calcd. for $C_{26}H_{32}O_8$: C, 66.07; H, 6.82. Found: C, 66.00, 65.91; H, 6.95, 6.90.

For titration the substance was dissolved in 0.5 ml. of acetone followed by 0.1 ml. of 0.5 *N* sodium acetate and 5 ml. of water. Under these conditions the nomilic acid, displacing acetic acid, remained in solution during the titration. The final end-point was reasonably stable, the faint pink of phenolphthalein fading only after several minutes. A weight buret was used with solutions standardized to weight-normality.

Nomilic acid (10.30 mg.) neutralized 0.466 g. of 0.0485 *N* NaOH. Nomilic acid (10.05 mg.) neutralized 0.443 g. of 0.0485 *N* NaOH. Equivalent weight, 477, 468. Calcd. for $C_{26}H_{32}O_8$, 472.5.

Hydrolysis of Nomilic Acid.—Nomilic acid was warmed on the steam-bath with an excess of 0.0485 *N* NaOH and titrated back with 0.0497 *N* HCl using phenolphthalein.

Nomilic acid (6.03 mg.) was dissolved in 1.440 g. of NaOH solution and back-titrated with 0.0896 g. of HCl solution. Nomilic acid (5.48 mg.) was dissolved in 1.211 g. of NaOH solution, back-titrated with 0.691 g. of HCl solution. Equivalent weight calcd. for 1 COOH, 1 lactone, 236. Found: 238, 224.

Isolation of Acetic Acid.—The aqueous solution from which the nomilic acid had separated (50 ml.) was steam distilled, the volume being maintained approximately constant. The volatile acid neutralized 8.1 g. of 0.0485 *N* NaOH. The neutral solution was concentrated to 2 ml. and the salt converted to the *p*-phenylphenacyl ester. On recrystallization from dilute alcohol and then petroleum ether the ester melted at 110–111° and gave no depression with an authentic specimen of the *p*-phenylphenacyl ester of acetic acid of the same melting point. The optical properties of the crystals were examined by Dr. Francis T. Jones and found identical for the two preparations.

Potassium Hydroxide Fusion of Nomilin.—Nomilin (62 mg.) was dissolved in a solution of 1 g. of potassium hydroxide in 1 ml. of water and gradually heated to 340° in a bath of Wood's metal. The distillate was collected in a solution of 2,4-dinitrophenylhydrazine sulfate. Nothing resembling acetone dinitrophenylhydrazone was observed, which in blank test was isolated in good yields.

Limonin Oxime.—Limonin (100 mg.) and 100 mg. of hydroxylamine hydrochloride were dissolved in 2 ml. of pyridine and 2 ml. of absolute alcohol and refluxed for four hours. The solvent was removed under reduced pressure and the residue washed well with water and recrystallized from dilute alcohol. The product decomposed and melted in the neighborhood of 260–270°, depending on the rate of heating. The product recrystallized from dilute ethanol appeared to hold that solvent very retentively. For analysis it was recrystallized twice from methanol and once from butanol and dried at 100° at 1 mm.

Anal. Calcd. for $C_{26}H_{31}O_8N$: C, 64.30; H, 6.43; N, 2.89. Found: C, 64.22, 64.04; H, 6.41, 6.30; N, 3.06.

Reaction of Nomilin with Hydroxylamine.—This was conducted as in the preparation of limonin oxime. However, nomilin oxime would not crystallize from any solvent or solvent mixture tried. An attempt to chromatograph the substance in a column of calcium carbonate resulted in decomposition. A preparation precipitated from methanol by the addition of water and dried at 100° at 1 mm. gave low C values. Calcd. for $C_{28}H_{35}O_9N$: C, 63.48; H, 6.67; N, 2.65. Found: C, 63.12; H, 6.63; N, 3.00. A sample precipitated from benzene with petroleum ether gave high C values; found C, 64.10; H, 6.80.

The hydroxy-hydroxamic acid which might conceivably result from the reaction of hydroxylamine with a lactone ring should be $C_{28}H_{37}O_{10}N$, requiring C, 61.39; H, 6.82.

Isolation of Substance X.—This substance separated in little aggregates from the alcoholic mother liquors of limonin from orange seeds. The yield of crude material from 22 lb. of orange seeds amounted to about 500 mg., but purification was accompanied by much loss on account of the difficulty of separating it from amorphous material. Solution in methanol followed by concentration or by dilution with water appeared the most effective method tried. The best preparation consisted of hair-like needles melting with profound decomposition at 315°.

For analysis the substance was dried to constant weight at 56° at 1 mm. Calcd. for $(C_9H_{12}O_4)_n$: C, 58.66; H, 6.57. Found: C, 58.62, 58.71; H, 6.55, 6.49.

Acknowledgment.—I wish to express my sincere thanks to Dr. W. C. Platt for the gift of the orange seeds.

Summary

Limonin was the sole bitter principle isolated from pulp and juice of Navel oranges. This substance was also obtained from seeds of lemons and Valencia oranges.

A new bitter principle, $C_{28}H_{34}O_9$, for which the name "nomilin" is proposed, was isolated from orange seeds and lemon seeds. Nomilin has a hydroxyl group that reacts with methylmagnesium iodide, but not readily with acetic anhydride. Like limonin, nomilin is a dilactone, but on treatment with dilute alkali followed by acidification acetic acid is lost and lactone acid, nomilic acid, $C_{26}H_{32}O_8$, is obtained. In contrast to limonin, nomilin does not yield appreciable amounts of acetone on fusion with potassium hydroxide. Limonin and nomilin both react with hydroxylamine in the presence of pyridine, limonin oxime being a beautifully crystalline substance.

The non-bitter precursor of limonin in Navel orange juice behaves like the diacid obtained by opening both lactone rings, but it is possible that the precursor may be a lactone acid or a compound of the diacid or lactone acid such as a glycoside.

ALBANY, CALIFORNIA RECEIVED AUGUST 21, 1947

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[20] I wish to express my sincerest thanks to Professor A. J. Haagen-Smit and Dr. G. Oppenheimer of California Institute of Technology for this determination.