of XVIII and XIX in a ratio of 1:9, respectively, as determined by the thin layer chromatography on silica gel G (Merck) using ether as the developing solvent. Recrystallization from acetone–hexane gave XIX as needles (6.23 g): mp 110–111°, $[\alpha]D$ +50° (*c* 0.75). This compound showed infrared bands at 5.60 (saturated γ-lactone) and 9.68 μ (N–O). Its nmr spectrum exhibited a singlet at τ 7.29, corresponding to two protons on the carbon atom adjacent to the lactone carbonyl group.

Anal. Calcd for $C_{13}H_{15}NO_3$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.69; H, 8.22; N, 5.86.

From the mother liquor of XIX there was obtained XVIII as needles (563 mg) after recrystallization from hexane: mp 135–136°, $[\alpha]D$ +63° (*c* 1.00). Its infrared spectrum showed bands at 5.61 (saturated γ-lactone) and 9.65 μ (N–O). The nmr spectrum exhibited a peak at τ 5.76 characteristic of the >CH–O grouping.

Anal. Calcd for $C_{13}H_{15}NO_3$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.72; H, 7.93; N, 5.84.

Further elution with methylene chloride–methanol (9:1) gave XI (3.24 g).

Catalytic Hydrogenation of Rearranged Tetrahydro-1,2-oxazolactone (XIX).—Compound XIX (5 g) in glacial acetic acid was hydrogenated with prereduced platinum oxide (500 mg) at room temperature and atmospheric pressure. Hydrogen (1 molar equiv) was taken up in 5 hr. Removal of the catalyst and evaporation of the solvent *in vacuo* gave an amorphous product (XX) (5.37 g). Part (50 mg) of this product in ethanol (1 ml) was treated with styphnic acid (50 mg) to yield a styphnate as fine crystals (72 mg): mp 214° dec (from acetone–ethanol), $[\alpha]D$ −21° (*c* 1.00, acetone). The infrared spectrum showed a band at 5.62 μ (saturated γ-lactone).

Anal. Calcd for $C_{19}H_{24}N_4O_{11}$: C, 47.11; H, 4.99; N, 11.57. Found: C, 47.37; H, 4.89; N, 11.42.

The amorphous product XX (200 mg) was dissolved in acetic anhydride (5 ml) and the solution was set aside at room temperature for 3 hr. Then pyridine (1 ml) was added and the mixture was allowed to stand at room temperature overnight. After acidification with 3% hydrochloric acid (100 ml), the product was isolated by extraction with methylene chloride. Crystallization from ethanol gave XXII as needles (153 mg): mp 237°, $[\alpha]D$ −125° (*c* 1.00). The infrared spectrum showed peaks at 2.95 (OH), 5.60 (saturated γ-lactone), and 6.16 μ (N-acetate).

Anal. Calcd for $C_{15}H_{23}NO_4$: C, 64.03; H, 8.24; N, 4.98. Found C, 63.84; H, 8.25; N, 4.82.

The mother liquor of XXII was concentrated *in vacuo* and the residue (70 mg) was chromatographed on alumina (5 g). Elution with methylene chloride yielded the N-acetate (20 mg), mp 170°, identified by direct comparison of an authentic sample of XXIII.

Elution with methylene chloride–methanol (95:5) gave XXII (24 mg), obtained above.

Elution with methylene chloride–methanol (from 9:1 to 1:1) afforded XXI as plates (18 mg): mp 251° (from methanol), $[\alpha]D$ −32° (*c* 1.00, methanol). This compound showed peaks at 2.95 (OH) and 6.15 μ (amide) in the infrared.

Anal. Calcd for $C_{13}H_{21}NO_3$: C, 65.24; H, 8.85; N, 5.85. Found: C, 64.93; H, 8.80; N, 5.71.

This compound was very sparingly soluble in the usual organic solvents except methanol and ethanol.

Base-Catalyzed Formation of Lactam Diol XXI.—The amorphous product XX (200 mg) obtained above was heated with pyridine (10 ml) at 100° for 1 hr. Evaporation of the solution *in vacuo* and crystallization of the residue from methanol afforded XXI (187 mg).

Dehydration of Hydroxy N-Acetate XXII.—Compound XXII (100 mg) was heated with pyridine and acetic anhydride (1:1, 5 ml) at 80° for 1 hr. The crude product (90 mg) obtained in the usual way was purified by chromatography on alumina (3 g). Elution with ether gave XXIII (78 mg).

Conversion of Lactam Diol-B XXI into N-Acetate XXIII.—Compound XXI (100 mg) was refluxed with pyridine–acetic anhydride (1:1, 5 ml) for 30 min. Removal of the solvent left a crystalline mass (98 mg), which, after crystallization from acetone, afforded XXIII (76 mg).

Catalytic Hydrogenation of Tetrahydro-1,2-oxazolactone-B (XVIII). **A.** Compound XVIII (150 mg) in glacial acetic acid (10 ml) was hydrogenated with prereduced platinum oxide (100 mg) at room temperature and atmospheric pressure for 7 hr. The uptake of hydrogen did not take place and the starting material (145 mg) was recovered.

B.—Compound XVIII (130 mg) in glacial acetic acid (10 ml) was hydrogenated in the presence of unreduced platinum oxide (500 mg) at room temperature and atmospheric pressure for 7 hr. During the hydrogenation, the mixture was vigorously stirred. The product (145 mg), isolated in the usual way, was acetylated by heating with acetic anhydride (3 ml) at 100°. After 1 hr, the mixture was poured into water and extracted with methylene chloride. The organic layer was washed with 3% aqueous sodium carbonate and water, dried over anhydrous sodium sulfate, and evaporated *in vacuo*, leaving a crystalline mass (155 mg). This was chromatographed on alumina (30 g) and elution with benzene afforded the starting material (15 mg). Elution with methylene chloride–methanol (9:1) yielded XXVI as plates (80 mg): mp 220–223° (from acetone), $[\alpha]D$ +28° (*c* 1.00). The infrared spectrum showed bands at 2.94 (OH), 5.77 (O-acetyl), 6.21 (amide), and 9.70 μ (C—O).

Anal. Calcd for $C_{15}H_{23}NO_4$: C, 64.03; H, 8.24; N, 4.98. Found: C, 64.21; H, 8.15; N, 5.01.

The nmr spectrum exhibited signals at τ 7.98 (3 H, O—$COCH_3$), 7.53 (2 H, singlet, CH_2 adjacent to the amide carbonyl group), 6.84 (1 H, OH), and 4.70 (1 H, multiplet, >CH—O). The signal at τ 6.84 disappeared upon addition of deuterium oxide.

Citrus Bitter Principles. VI.[1] Ichangin

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The isolation and structure determination of ichangin, a new C_{26} terpenoid bitter principle from a hybrid of *Citrus ichangensis*, are described. Ichangin is assigned structure **3** and is closely related structurally to limonin.

From the viewpoint of their limonoid content, the genus *Citrus* (Rutaceae) is a very homogeneous group.[1] Examination of the bitter principles in the seed extracts of different species revealed that one of the most abnormal members of this genus was *Citrus ichangensis* and its hybrids.[1,3] *C. ichangensis* and its hybrids showed a remarkable ability to accumulate relatively large amounts of limonin (**1**) intermediates. Seed extracts from these sources on thin layer chromatography (tlc)[4] showed a much more complex pattern of components, containing four to five other limonoid spots, in addition to the usual obacunone,[5,6] limonin,[6] nomilin,[6,7] and deacetylnomilin.[4]

(1) Part V: D. L. Dreyer, *Phytochemistry*, in press.

(2) A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

(3) For a detailed discussion of the botany of the genus *Citrus*, see W. T. Swingle, "The Citrus Industry," Vol. 1, H. J. Webber and L. D. Batcheler, Ed., University of California Press, Berkeley, Calif., 1943, p 386.

(4) D. L. Dreyer, *J. Org. Chem.*, **30**, 749 (1965).

(5) T. Kubota, T. Matsuura, T. Tokoroyama, T. Kamikawa, and T. Matsumoto, *Tetrahedron Letters*, 325 (1961); O. H. Emerson, *J. Am. Chem. Soc.*, **73**, 2621 (1951).

(6) D. H. R. Barton, S. K. Pradhan, S. Sternhell, and J. F. Templeton, *J. Chem. Soc.*, 255 (1961).

(7) O. H. Emerson, *J. Am. Chem. Soc.*, **70**, 545 (1948).

TABLE I

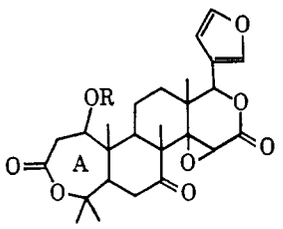
NMR SPECTRA OF LIMONIN AND OBACUNONE DERIVATIVES IN TRIFLUOROACETIC ACID[a]

	α furan	β furan	H-17	H-15	C-methyls				
Obacunoic acid (**6**)	445	383	340	238	97	96	93	85	73
Obacunone (**5**)	443	381	337	246	99	86	76	73	70
Nomilin (**2b**)	444	382	339	244	101	90	89	82	74
Deacetylnomilin (**2a**)	447	384	343	244	102	94	87	82	74
Limonin (**1**)	444	382	341	257		87	80	78	76
Ichangin (**3**)	447	384	342	233		88	88	84	73

[a] See footnote 15.

One of these minor bitter materials, named ichangin, has now been isolated from ichang lemon seeds (*C. ichangensis* × *C. grandis*?) and its presence in yuzu (*C. ichangensis* × *C. reticulata*) and *C. ichangensis*[8] seeds was indicated by tlc. Extracts of ichang lemon seeds from early season fruit proved to be one of two cases of a Citrus species or hybrid yet examined where limonin was not the major bitter principle.[1] In these cases greater amounts of deacetylnomilin (**2a**) were obtained.

Ichangin was initially detected by tlc and was isolated by chromatography on acid-washed alumina of the mother liquors remaining after separation of the bulk of the limonin (**1**), nomilin (**2b**), and deacetylnomilin (**2a**) by crystallization. Ichangin (**3**), mp 209–212°,



2a, R = H
b, R = Ac

analyzed for $C_{26}H_{32}O_9$ and tasted bitter. The material showed only end adsorption in the ultraviolet. The infrared spectrum showed hydroxyl adsorption at 3630 and 3270 cm^{-1}, a single, broadened, high-intensity, carbonyl band, and characteristic bands assigned to a β-substituted furan ring.[9]

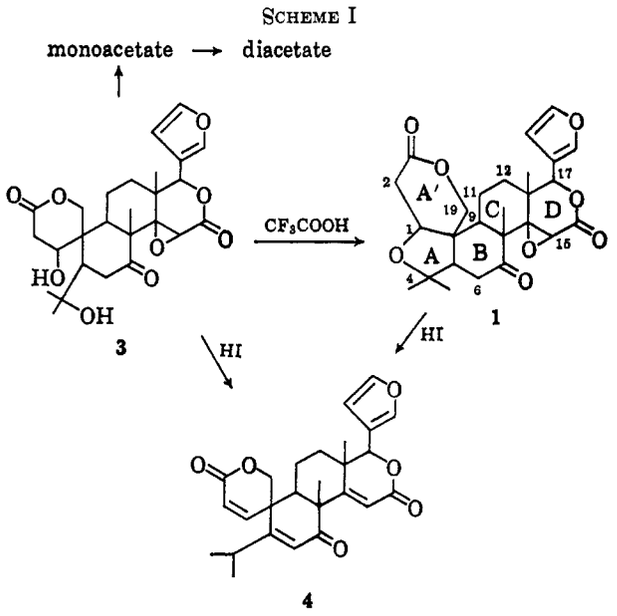
The nmr spectrum of **3** was similar to that of limonin (**1**)[10] and showed bands assigned to a β-substituted furan ring, a furfurylic proton at C-17, an epoxy proton at C-15, an AB quartet assigned to a C-19 methylene group, and four C-methyl groups (Table I). In addition, there was a triplet at 268 cps which integrated for one proton.[11] This resonance was assigned to a carbinol proton and the splitting pattern indicated an equatorial proton with one adjacent axial and one adjacent equatorial proton. The position of the epoxy H-15 resonance was similar to that in **2a**, **2b**, or similar systems having an open or unstrained A-ring, but unlike limonin derivatives having a five-membered A-ring. Such strained five-membered A-rings cause the H-15 resonance to occur 10–30 cps further downfield.[10] As can be seen from Table I, the H-15 resonance in **3** occurs at the high-field end of the range. The multiplicity of the furan resonances in **3** showed that the D-ring is identical with that in the other citrus bitter principles. The downfield position of the C-methyl resonances in **3** suggested that there must be a single-bond oxygen function at C-4[12] as in the other limonoids (Table I).

The ORD curve of **3** showed a negative Cotton effect, similar in profile and position with those from the other citrus limonoids.[4]

The sum of the above spectroscopic data suggests that the B- and D-rings of ichangin are identical with those in limonin (**1**).

Ichangin was recovered unchanged upon acidification after treatment with dilute base. Acetylation of ichangin (**3**) (Scheme I) in acetic anhydride in pyridine

SCHEME I



gave a mono- and a diacetate. Acetylation with acetic anhydride–sodium acetate gave seven products (examined by tlc) in about equal amounts, one of which corresponded to limonin (**1**). Citrolin (**4**) was obtained from **3** with hydriodic acid under the same conditions used to obtain it from limonin (**1**).[6,13] The formation of **4** indicates that **3** must have a limonin carbon skeleton, and furthermore, the hydroxyl groups cannot be located at the 9, 11, or 12 positions. Location of a hydroxyl group at the 6 position is excluded by the ORD curve of **3** which is too close to that of limonin[4]

(8) Seeds of *C. ichangensis* are quite rare and only *ca.* 5 g of seeds was available.

(9) T. Kubota, *Tetrahedron*, **4**, 68 (1958).

(10) D. L. Dreyer, *ibid.*, **21**, 75 (1965).

(11) This triplet (in trifluoroacetic acid) was a quartet in dimethyl sulfoxide–deuteriochloroform centered at 234 cps with ratios 1:3:3:1. Of the two hydroxyl protons, one appeared to be a doublet which was partly overlapped with the β-furan resonance. The other was present as a singlet in this solvent system at 348 cps. The position and sharpness of both the hydroxyl resonances were temperature dependent. See O. L. Chapman and R. W. King, *J. Am. Chem. Soc.*, **86**, 1256 (1964).

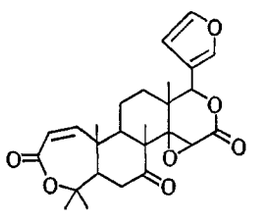
(12) See, *e.g.*, S. Ito, M. Kodama, T. Nozoe, H. Hikino, Y. Hikino, Y. Takeshita, and T. Takemoto, *Tetrahedron Letters*, 1787 (1963).

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

for an α-hydroxyl group to be present.[14] Location of a hydroxyl group at the 6 or 2 positions is also excluded by the chemical shift of the carbinol proton which was too far upfield to be α to a carbonyl group.

The 1 and 4 positions are thus indicated for the location of the two hydroxyl groups. The position of the H-15 resonance is consistent with an open A-ring, and most known limonoids from the Rutaceae differ only in structure of the A-ring. Ichangin (**3**) could be derived biogenetically in a simple manner from deacetylnomilin (**2a**) by oxidative cyclization onto the C-19 methyl group. Formation of the diacetate could occur by a intramolecular acyl transfer. Treatment of **3** with trifluoroacetic acid in glacial acetic acid gave an anhydro derivative whose physical properties were identical in all respects with those of limonin (**1**). Structure **3** is thus indicated for ichangin.

Previous circumstantial evidence[4] suggested that the route, deacetylnomilin → nomilin → obacunone → obacunoic acid → isoobacunoic acid → limonin, is operating in the final stages of the biogenesis of limonin. The isolation of ichangin and its *in vitro* conversion to limonin suggests that the route deacetylnomilin → ichangin → limonin must be considered as a possible alternative biogenetic route to limonin.



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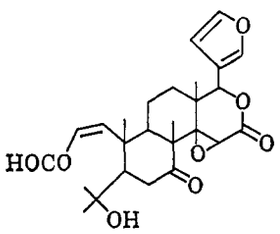

6

The large buildup of **3** in *C. ichangensis* hybrids appears to result from the large pool of **2a** available and the nonspecificity, in terms of substrate structure, of the enzymes involved.

Experimental Section[15]

Isolation of Ichangin (3).—The residue from the acetone extracts of ground, defatted, ichang lemon seeds was repeatedly crystallized to give **1**, **2a**, and **2b** as described previously.[4] From 5 kg of defatted seeds 22 g of **2a**, 11.5 g of **2b**, and 1.5 g of **1** were obtained. All mother liquors from these operations were combined and the solvent was removed. The residue was taken up in chloroform and the chloroform solution washed with 5% sodium bicarbonate. The solvent was removed from the neutral fraction and the residue was taken up in benzene and chromatographed on acid-washed alumina. Elution with benzene gave fractions containing obacunone. Elution with chloroform gave limonin and nomilin. Elution with 10% acetone in chloroform gave fractions containing ichangin; further elution gave deacetylnomilin. The fractions were monitored by the tlc system described previously.[4] Those fractions rich in ichangin were combined and solvent was removed. The residue was crystallized from benzene–ethanol: mp 205–208°. Recrystallization from methanol gave mp 209–212°; R_f 0.65 that of limonin; infrared, ν 3630 and 3270 (hydroxyl), 1739 (carbonyl), 1600 (unassigned), 1504 and 880 (β-substituted furan) cm^{-1} (Nujol); $\lambda_{max}^{95\% EtOH}$ 203 mμ (ϵ 8500); nmr, δ 451 (d) J = 1 (α furan), 387 (t) J = 1 (β furan), 330 (s) (H-17), 305, 287 (AB) (d) J = 14 (H-19), 234 (q) $J_{a,e}$ = 6, $J_{e,e}$ = 5 (H-1), 217 (s) (H-15), 80, 78, 75, 66 (C-methyls) cps (dimethyl sulfoxide–deuteriochloroform); ORD in dioxane (c 0.085) at 27°, $[\alpha]_{600}$ −78.6°, $[\alpha]_{315}$ −1180°, $[\alpha]_{309}$ −988°, $[\alpha]_{305}$ −1060°, $[\alpha]_{296}$ −588° (sh), $[\alpha]_{285}$ −120° (sh), $[\alpha]_{278}$ −24°, $[\alpha]_{253}$ −920°, $[\alpha]_{230}$ +1150° (last reading).

Anal. Calcd for $C_{26}H_{32}O_9 \cdot 0.5CH_3OH$: C, 63.0; H, 6.79. Found: C, 62.9, 62.8; H, 6.65, 6.55.

Acetylation of Ichangin.—Ichangin was allowed to stand overnight with acetic anhydride–pyridine. After work-up the crude product was chromatographed over acid-washed alumina to give, on elution with benzene–chloroform, a diacetate: mp 246–248.5° (from acetone–methanol); infrared, ν 1739, 1720 (carbonyl), 1500, 881 (β-substituted furan) cm^{-1} (Nujol); nmr, δ 448 (d) J = 1 (α furan), 383 (t) J = 1 (β furan), 329 (s) (H-17), 224 (s) (H-15), 130, 122 (acetyl), 92, 92, 69, 65 (C-methyls) cps (deuteriochloroform). The amount of material obtained was too small for complete characterization.

Further elution of the column with chloroform gave ichangin monoacetate: mp 147–151° (from methanol); infrared, ν 1710, 1740 (carbonyl) 1500, 873 (β-substituted furan) cm^{-1} (Nujol); nmr, δ 444 (α furan), 382 (β furan), 328 (H-17), 216 (H-15), 130 (acetate), 80, 76, 73, 65 (C-methyls) (deuteriochloroform).

Anal. Calcd for $C_{28}H_{34}O_{10}$: C, 63.38; H, 6.46. Found: C, 62.7; H, 6.64.

Citrolin (4).—Ichangin was refluxed with 1:1 glacial acetic acid–hydriodic acid for 40 min. The mixture was diluted with water and extracted with chloroform. The chloroform layer was washed, dried, and concentrated. The solution was filtered through a short column of acid-washed alumina with chloroform. The product, mp 292–295°, had R_f values and infrared and ultraviolet spectra identical with those of citrolin prepared from limonin.[6,13]

Limonin (1).—Ichangin was warmed on a steam bath with trifluoroacetic acid in glacial acetic acid for 2 hr. The reaction mixture was diluted with water and was extracted with chloroform to give, after drying and removal of solvent, a residue which was crystallized from methylene chloride–2-propanol: mp 296°. The nmr, infrared, and ultraviolet spectra, ORD, melting point, R_f on tlc, and solubility properties were identical with those of limonin.

Acknowledgment.—The author is indebted to Norman Mitchell of Applied Physics Corporation, Monrovia, California, for the ORD curves and to Ed Nauer, Citrus Research Center, University of California, Riverside, California, for citrus fruit.

(14) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p 111.

(15) Nmr data were obtained at 60 Mc and are given in cycles per second relative to internal tetramethylsilane. The relative area of peaks was consistent with the assignments.