Citrus Bitter Principles. III. Isolation of Deacetylnomilin and Deoxylimonin

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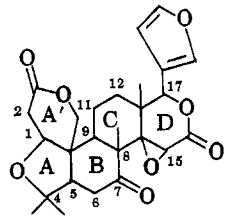
Deacetylnomilin has been isolated from four *Citrus* species and *Poncirus trifoliata.* Its structure was shown by spectroscopic studies and conversion to obacunone and nomilin. The role of deacetylnomilin in the biogenesis of limonin is discussed. It is very probable that deacetylnomilin is identical with the previously isolated isolimonin. Deoxylimonin has been isolated from *C. paradisi. P. trifoliata* also proved to be a good source of the coumarins, imperatorin and bergapten.

During a phytochemical survey of bitter materials in seeds of the genera *Citrus* and *Poncirus* (Rutaceae), by means of thin layer chromatography (t.l.c.), a minor spot was observed from some species. The R_f-value of this spot, about 0.5 that of limonin, did not correspond to that of any of the known C_{26} bitter principles. On t.l.c. the spot gave a positive Ehrlich's test,[2] which constitutes good evidence for the presence of a furan ring in the substrate. Limonoids give characteristic off-colored orange spots with this reagent.

The new bitter principle was isolated on a preparative scale from seeds of *Citrus aurantium* Linn., *C. sinensis* (Linn.) Osbeck (navel orange seeds), *C. grandis* (Linn.) Osbeck, *C. paradisi,* Ichang lemon (hybrid; *C. ichangensis* × *C. grandis*), and *Poncirus trifoliata.* Different batches of seeds tended to vary in their content of coumarins and especially the bitter principles. Limonin (I),[3] however, was always the major bitter principle isolated. The new compound was obtained by chromatography of acetone extracts of defatted seeds on acid-washed alumina after the bulk of the limonin had been removed by crystallization. It was obtained from Ichang lemon seeds without resort to chromatography, as described in the Experimental section.



I

The new material, m.p. 263–265°, showed a correct analysis for $C_{26}H_{32}O_8$. It showed only high-intensity end absorption in the ultraviolet and had a hydroxyl and a single broadened high-intensity carbonyl band in the infrared. Characteristic infrared bands assigned to a β-substituted furan were also present.[4] The n.m.r. spectrum was grossly similar to that of the other citrus bitter principles.[5] The spectrum contained five C-methyl resonances which were very similar in pattern to those shown by nomilin (II) or obacunone (III). Thus, the new compound was related to obacunone (III) rather than to limonin (I) which has only four C-methyl groups. As is the case with nomilin and obacunone one of the downfield C-methyl resonances was strikingly broad. In addition, the n.m.r. spectrum had bands which were assigned to (a) a β-substituted furan ring, (b) a furfurylic proton at H-17, and (c) an epoxy proton at H-15. There was also a complex multiplet centered at about δ = 4.2 p.p.m. which integrated for one proton. Previous studies[5] on the n.m.r. spectra of the citrus bitter principles have shown that the observed chemical shifts are especially sensitive to changes in structure. The chemical shift for the H-15 proton is quite dependent upon the nature of the group at C-7 and on the type of A-ring present, *e.g.,* a fused 5- or 7-membered ring. When the A-ring is 5-membered, the B-ring is flattened somewhat causing a change in the position of the C-7 keto group with respect to the H-15 proton. This causes the position of the H-15 resonance in the limonin series to be 20–30 c.p.s. downfield from its position in the obacunone series. The position of this resonance in the new bitter material at 227 c.p.s. (in chloroform–dimethyl sulfoxide) suggested absence of the fused tetrahydrofuran A-ring of the type occurring in limonin (I). The multiplicity of the furan resonances is very sensitive to the nature of the D-ring. For example, replacement of the epoxy group by a double bond causes a great increase in the complexity of the furan resonances. The furan resonances in the new bitter principle had exactly the same splitting pattern as obacunone or limonin. Thus, on the basis of its n.m.r. spectrum the D-ring of the new compound is identical with the D-ring in obacunone (III) or limonin (I) and the C- and B-rings are very probably identical with those in III or I.

Acetylation of the new compound gave two products which were separated by preparative thin layer chromatography and proved to be identical with obacunone (III) and nomilin (II).

The structure of nomilin (II) rests largely upon its n.m.r. spectrum[5] and its conversion to obacunoic (V) and acetic acids under basic conditions[6] or to obacunone (III) with acetic anhydride and pyridine.[7,8] It follows from these conversions that the new compound must be the deacetyl derivative of nomilin (IV).

Attempted hydrolysis of II to IV under mild acidic conditions gave instead only III. Deacetylnomilin (IV) proved to be remarkably stable to base. Repeated attempts to convert IV to V, under conditions in which obacunone[3] or nomilin[6] are smoothly converted to obacunoic acid (V), resulted in the complete re-

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[1] A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

[2] *p*-Dimethylaminobenzaldehyde followed by treatment with hydrogen chloride gas: T. Reichstein, *Helv. Chim. Acta,* **15**, 1110 (1932).

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

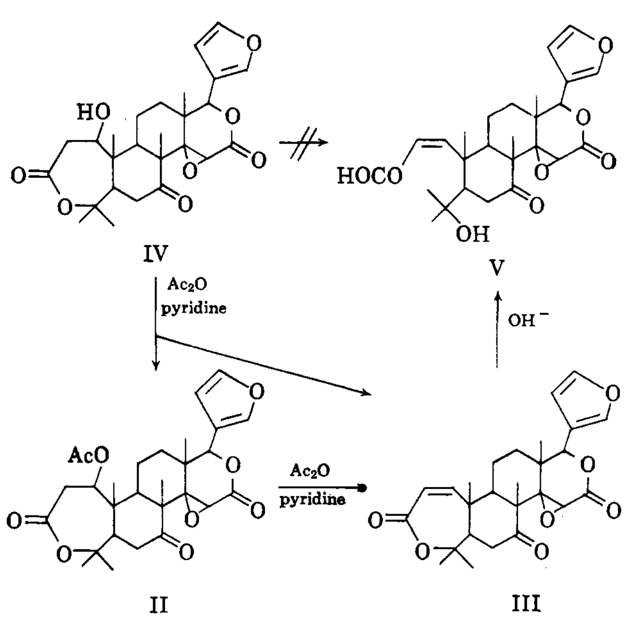
[4] See for example footnote 24 in A. Melera, K. Schaffner, D. Arigoni, and O. Jeger, *Helv. Chim Acta,* **40**, 1420 (1957).

[5] D. L. Dreyer, *Tetrahedron,* **21**, 75 (1965).

[6] O. H. Emerson, *J. Am. Chem. Soc.,* **70**, 545 (1948).

[7] O. H. Emerson, *ibid.,* **73**, 2621 (1951).

[8] F. M. Dean and T. A. Geissman, *J. Org. Chem.,* **23**, 596 (1958).

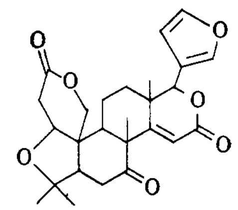


covery of starting material (IV). Thus, elimination of the 1-hydroxyl group occurs only *via* the acetyl derivative (IV) possibly through an intramolecular process.

In spite of some confusion in the literature, it appears very probable that deacetylnomilin (IV) is identical with isolimonin. Isolimonin has been isolated from various *Citrus* species and was first reported by Koller and Czerny[9] and later by Higby.[10,11] The physical properties previously reported[9-11] are close to those found in this investigation. Isolimonin has been reported[9] to give 2 moles of methane with methyl Grignard, whereas nomilin gives only 1 mole.[6] The formation of methane from hindred enolizable ketones with methyl Grignard as in the case of the 7-keto group of obacunone is well documented.[12] These results are consistent with isolimonin being identical with deacetylnomilin.

The optical rotatory dispersion (O.R.D.) curves of limonin, nomilin, and deacetylnomilin are almost identical in profile and exhibit a negative multiple Cotton effect curve with the first trough occurring at 317, 315, and 314 mμ, respectively. The similarity of the O.R.D. curve of limonin with that of 7-ketocholestanyl acetate has previously been pointed out.[3,13] The similarity of the O.R.D. data testifies to the stereochemical homogeneity of this series of compounds about the B-ring.

Grapefruit (*C. paradisi*) seed extracts, when worked up on a large scale (30–40 kg.), showed a further "bitter-type spot" between limonin and deacetylnomilin. Extensive chromatography over alumina of the acetone extracts, after removal of the limonin by crystallization, gave a product, m.p. 323–325°, which proved to be identical with a synthetic sample of deoxylimonin (VI).[3,14]



VI

This is the second report of a previously synthetic limonoid derivative in the Rutaceae. Hirose[15] reported in 1963 that evodol (from *Evodia rutaecarpa*) was identical with limonin diosphenol, a compound prepared during work on the structure of limonin.[3]

Emerson reported[7] obacunone to be tasteless, a result in which we concur. Nomilin is quite bitter, but the deacetyl derivative (IV) is again tasteless.[16] Deoxylimonin also proved to be tasteless.

The failure of deacetylnomilin to eliminate water, whereas nomilin easily eliminates acetic acid and fails to undergo hydrolysis of the acetoxy group, may have some biogenetic implications. Assuming that these observations reflect to some degree the processes occurring in the plant, the sequence of steps leading to limonin (I) would be elimination of the 1-hydroxyl group of IV through the acetate (II) to give obacunone (III), which could then give limonin (I) by a Michael addition and oxidation onto the C-19 angular methyl group. We regard the origin of deoxylimonin (VI) to be a result of incompleteness of the epoxidation step in the biogenesis of limonin rather than reduction of limonin to deoxylimonin. Most of the products of the Rutaceae are the result of extensive oxidation and few show any evidence of reductive steps in their biogenesis. If deoxylimonin arises in this manner one might expect to find deoxy derivatives of the other naturally occurring limonoids, for example, deoxyobacunone or deoxynomilin.

Experimental[17]

Deacetylnomilin (IV).—The dried and ground seeds were defatted with petroleum ether. In the case of *P. trifoliata*, cooling the petroleum ether extracts resulted in a deposit of crystalline material which was collected and recrystallized from ethyl acetate–petroleum ether to give imperatorin: m.p. 95–97°; ν 1739, 1722 cm.$^{-1}$ (Nujol); n.m.r., δ = 6.26 (d, J = 10 c.p.s., H-3), 7.70 (d, J = 10 c.p.s., H-4), 7.29 (s, H-5), 6.78 (d, J = 2 c.p.s., H-6), 7.62 (d, J = 2 c.p.s., H-7), 4.93 (d, J = 8 c.p.s., allyl methylene), 5.55 (t, J = 7 c.p.s., vinyl), 1.70 (s, γ-methyls) p.p.m. ($CDCl_3$). The ultraviolet[18,19] and infrared curves were identical with those from an authentic sample isolated from grapefruit oil.[19]

Combination of the mother liquors with further crops of material from the original petroleum ether extracts, work-up by chromatography over alumina, and crystallization from ethanol gave 5-methoxypsoralen (bergapten): ν 1752 cm.$^{-1}$ (Nujol); n.m.r., δ = 6.25 (d, J = 10 c.p.s., H-3), 8.14 (d, J = 10 c.p.s., H-4), 7.03 (d, J = 2 c.p.s., H-5), 7.61 (d, J = 2 c.p.s. H-7), 7.09 (s, H-8), 4.27 (s, methoxy) p.p.m. ($CDCl_3$). The ultraviolet and infrared curves were identical with those obtained from an authentic sample.[7]

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(9) G. Koller and H. Czerny, *Monatsh.*, **67**, 248 (1936).

(10) R. H. Higby, *J. Am. Chem. Soc.*, **60**, 3013 (1938).

(11) See also, A. Mookerjee, *J. Indian Chem. Soc.*, **17**, 593 (1940); T. R. Seshadri and J. Veeraraghaviah, *Proc. Indian Acad. Sci.*, **11A**, 505 (1940); *Chem. Abstr.*, **34**, 8090 (1940); D. Nomura, *Hakko Kogaku Zasshi*, **30**, 199 (1952); *Chem. Abstr.*, **47**, 791 (1953).

(12) See for example, F. Runge, "*Organometallverbindungen*," Wissenschaftliche Verlagsgesellschaft M.B.H., Stuttgart, 1944, p. 383.

(13) D. Arigoni, *et al.*, *Experientia*, **16**, 41 (1960).

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

(15) Y. Hirose, *Bull. Pharm. Soc. Japan*, **11**, 535 (1963).

(16) There is perhaps some generality in the observation that acetylation will sometimes give bitter derivatives. For example, the acetyl derivatives of glucose and sucrose are somewhat bitter.

(17) N.m.r. data were obtained at 60 Mc. and are given in δ relative to internal tetramethylsilane. The relative areas of peaks were consistent with the assignments.

(18) Y. N. Sharma, A. Zaman, and A. R. Kidwai, *Tetrahedron*, **20**, 87 (1964).

(19) W. L. Stanley and S. H. Vannier, *J. Am. Chem. Soc.*, **79**, 3488 (1957).

The petroleum ether extracts from seeds of the genus *Citrus* were relatively poor in coumarins and contained mostly fats. The defatted seeds were extracted with acetone, solvent was removed, and the residue was crystallized from methylene chloride–isopropyl alcohol to give limonin. Solvent was then removed from the mother liquors; the residue was taken up in benzene and chromatographed over a column of acid-washed alumina made up with benzene. Elution with chloroform gave fractions containing mostly limonin. Elution with 1:1 acetone–dichloroethane or acetone–chloroform gave fractions containing deacetylnomilin. The fractions were monitored by means of t.l.c. using silicic acid. The plates were developed with 1:1 chloroform–ethyl acetate. The spots were detected by spraying the plates with 5% *p*-dimethylaminobenzaldehyde in ethanol and immersing them in a closed vessel of hydrogen chloride gas.[2,20] Obacunone had the highest R_f and was cleanly separated from limonin. Nomilin and limonin have about the same R_f and their spots can or cannot be resolved depending upon the brand of adsorbent. Deacetylnomilin has an R_f *ca.* one-half that of limonin.

The recovery of material from the column was not always good and seemed to be somewhat variable. The variations were presumably due to some opening of the lactone rings on the column. In some runs, upon standing, the deacetylnomilin would crystallize from those fractions in which it was present in high concentrations. In general, however, the fractions that contained deacetylnomilin were pooled, solvent was removed, and the residue was crystallized from ethanol or benzene: m.p. 259–261°.

Deacetylnomilin was obtained as follows without chromatography. The acetone extracts of defatted seeds of Ichang lemon were concentrated and a precipitate of crude limonin was obtained. Complete removal of solvent from the mother liquors gave a residue which was washed with hot methylene chloride. The methylene chloride solution was filtered from the residue, isopropyl alcohol was added, and the solution was concentrated giving two crops of limonin. The methylene chloride insoluble fraction was warmed with more methylene chloride and filtered, and isopropyl alcohol was added to give a crop of limonin–nomilin mixture and a second crop of almost pure nomilin. The methylene chloride insoluble material thus remaining consisted of almost pure deacetylnomilin: m.p. 253–260°; recrystallization from ethyl acetate gave m.p. 263–265°; ν 3500 (hydroxyl), 1746 (carbonyl), 1513, and 879 (β-substituted furan) cm.$^{-1}$ (Nujol); n.m.r., δ = 7.45 (d, J = 1 c.p.s., α-furan), 6.40 (t, J = 1 c.p.s., β-furan), 5.72 (s, H-17), ~4.2 (complex multiplet, H-1),[21] 4.07 (s, H-15), 1.70, 1.57, 1.45, 1.38, 1.23 (C-methyls) p.p.m. (trifluoracetic acid). O.R.D. in dioxane (*c* 0.17) at 27°: $[\alpha]_{600}$ −112°, $[\alpha]_{314}$ −1530°, $[\alpha]_{309}$ −1365° $[\alpha]_{305}$ −1436°, $[\alpha]_{296}$ −895° (sh), $[\alpha]_{286}$ −353° (sh), $[\alpha]_{278}$ −200°, $[\alpha]_{270}$ −435°.

Anal. Calcd. for $C_{26}H_{32}O_8$: C, 66.08; H, 6.83. Found: C, 66.1; H, 6.83.

Nomilin (II).—O.R.D. in dioxane (*c* 0.22) at 27°: $[\alpha]_{600}$ −91°, $[\alpha]_{315}$ −564°, $[\alpha]_{310}$ −510°, $[\alpha]_{306}$ −520°, $[\alpha]_{297}$ −310° (sh), $[\alpha]_{287}$ −118° (sh), $[\alpha]_{279}$ −45°, $[\alpha]_{270}$ −273°.

Limonin (I).—O.R.D. in dioxane (*c* 0.18) at 27°: $[\alpha]_{600}$ −116°, $[\alpha]_{317}$ −1850°, $[\alpha]_{312}$ −1630°, $[\alpha]_{308}$ −1657°, $[\alpha]_{298}$ −885° (sh), $[\alpha]_{290}$ −193° (sh), $[\alpha]_{280}$ +138°, $[\alpha]_{274}$ 0°.

Acetylation of Deacetylnomilin.—Deacetylnomilin was acetylated by refluxing with acetic anhydride–pyridine for 2 hr. The reaction mixture was decomposed with water and the products were extracted with chloroform. After removal of solvent, the residue was subjected to preparative t.l.c. on the same system as that used for monitoring the bitter principles. In this manner, obacunone and nomilin were isolated. These materials gave infrared curves which were identical with those obtained from authentic samples.

Attempted Hydrolysis of Nomilin.—A dioxane–hydrochloric acid solution of nomilin was allowed to stand for several days at room temperature. Isolation of the bitter principles with chloroform and spotting on t.l.c. as above showed obacunone as the only product.

Deoxylimonin (VI).—Grapefruit seed meal, from which most of the oil had been expressed, was defatted with hexane and then extracted with acetone. Solvent was removed from the acetone extracts and the residue was crystallized from methylene chloride–isopropyl alcohol to give limonin. Solvent was removed from the mother liquors and the residue was stirred into a large volume of hexane to further remove hexane-soluble fats and waxes. The insoluble material was collected and chromatographed on acid-washed alumina with benzene. Small amounts of unidentified coumarins were first eluted, followed by obacunone. Limonin was eluted with 1:4 benzene–chloroform, deoxylimonin with 1:4 acetone–chloroform, and deacetylnomilin with 1:2 acetone–chloroform. Those fractions rich in deoxylimonin were pooled and rechromatographed or, if especially pure, crystallized from acetone–ethanol to give the product of m.p. 323–325°. The yield from 25 kg. of seed meal was *ca.* 1 g. The natural material gave no depression in mixture melting point with a synthetic sample[3,14]; infrared spectrum and R_f on t.l.c. (0.60 that of limonin) were identical with those from a synthetic sample.

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(20) A desiccator provided with an evacuation valve to which a hydrogen chloride tank can be attached works very well for such purposes.

(21) This multiplet is partly overlapped with the epoxy, H-15, resonance.