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ISOLIMONIC ACID, A NEW CITRUS LIMONOID

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Key Word Index—*Citrus aurantium*; *C. reticulata*; *C. paradisi*; Rutaceae; limonoids; isolimonic acid; ichangin.

Abstract—Isolimonic acid was isolated as its methyl ester from seeds of *Citrus aurantium*, *C. reticulata* and *C. paradisi*, and its structure was determined by ^{1}H and ^{13}C NMR. The isomeric limonoid ichangin was also isolated from *C. aurantium* and *C. paradisi*.

INTRODUCTION

In our previous work on acidic limonoids of grapefruit (*Citrus paradisi*) seeds we isolated a compound whose ^{1}H NMR spectrum indicated that it was not closely related to any of the known citrus limonoids [1]. However, it was present in very low concentration relative to the other acidic limonoids and the amount obtained was insufficient for a definitive structure determination. We have now found this same compound in high concentration in sour orange (*C. aurantium*) seeds, and we have determined its structure.

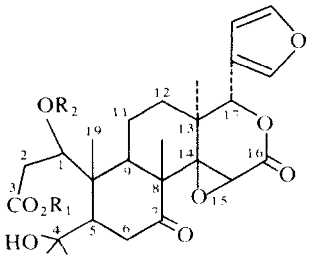
RESULTS AND DISCUSSION

An extract of sour orange seeds was separated into acidic and neutral fractions. The acidic fraction was methylated to facilitate separation of its components by chromatography. TLC showed three spots giving the characteristic colour reaction for limonoids with Ehrlich's reagent [2]. The two major spots corresponded in mobility to the methyl ester of the compound previously isolated from grapefruit seeds and to methyl deacetylnomilinate (**2**), while the minor one corresponded to methyl nomilinate (**4**). The methyl ester of the new compound, which we have named isolimonic acid (**5**), was isolated by column chromatography.

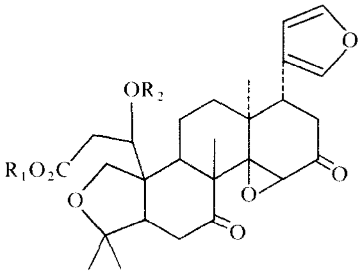
Accurate mass measurement by MS gave an empirical formula of $C_{27}H_{34}O_9$ for methyl isolimonate (**6**). The ^{1}H NMR spectrum of **6** in $CDCl_3$ showed signals characteristic of a normal limonoid D-ring, i.e. a six-membered lactone and 14,15-epoxide. This, with the furan ring, accounts for four of the nine oxygens in the empirical formula and the methyl ester group for two more, leaving three in question. The ^{13}C NMR spectrum showed the lactone and ester carbonyls and in addition a ketone carbonyl. Seven resonances were observed in the region of the spectrum ascribable to carbons singly bonded to oxygen. Four of these can be assigned to the methyl ester, epoxide and lactone groups, leaving three carbons, resonating at δ 68, 74, and 83, attached to the remaining two oxygens. Therefore, one of these oxygens must be part of a cyclic ether system and the other must be a hydroxyl. Selective heteronuclear decoupling showed that the signal at δ 74 was coupled to a one-proton multiplet at δ 4.18 in the ^{1}H NMR spectrum. Upon acetylation of **6** this signal moved downfield to 5.52 in **7**, and thus it represents a proton on a carbon atom bearing a secondary alcohol group. The two ether carbons were shown by the SFORD ^{13}C NMR spectrum to be quarternary (83 ppm) and methylene (68 ppm). The latter was coupled to an AB quartet in the proton spectrum; in $CDCl_3$ this quartet was partially obscured by the H-15 and methyl ester resonances, but in C_6D_6–DMSO-d_6 the AB system showed clearly. The position of these signals (around 4 ppm) and the coupling constant (11 Hz) were similar to those of the H-19 resonances in atalantin (**8**) [3], which contains a 4,19-cyclic ether system. All of the known citrus limonoids are oxygenated at C-4, and those that contain only four quarternary methyl groups, as does **5**, are all oxygenated at C-19. Therefore, a 4,19-cyclic ether structure was assigned to **5**.

The positions of the secondary hydroxyl and ketone groups remained to be established. The chemical shift of the H-15 resonance in limonoids depends upon the nature of the substituent at the 7-position, because of the close spatial proximity of the two sites [4]. The H-15 signal was not affected when **6** was acetylated; the secondary alcohol could not then be at the 7-position, since H-15 undergoes a strong upfield shift when either 7α- or 7β-limonol is acetylated [4]. However, the downfield location of the H-15 resonance showed the presence of an oxygenated function at C-7, and therefore the ketone is assigned to this position.

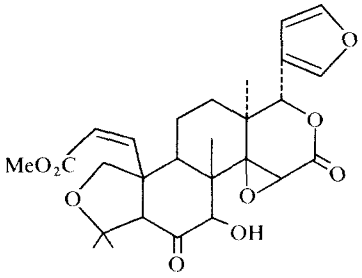
Since C-1 is either oxygenated or unsaturated in limonoids, and the ^{13}C NMR spectrum of **6** showed no olefinic carbons, the secondary alcohol group was most reasonably assigned to the 1-position. This assignment was substantiated by the behavior of the acetate **7** when treated with base. The acetate group was eliminated and a double bond was formed. The ^{1}H NMR spectrum of the product (**9**) showed a widely separated olefinic AB quartet, characteristic of a double bond conjugated to a carbonyl group. The large coupling constant (16 Hz) indicated a *trans* configuration of the double bond, rather than *cis* as in atalantin (**8**). Thus, the NMR spectral data were consistent with the structure **5** for isolimonic acid. The only other limonoids known to contain 4,19-cyclic ether groups are those of *Atalantia* [3], a genus closely related to *Citrus*.



1 $R_1 = R_2 = H$
2 $R_1 = Me, R_2 = H$
3 $R_1 = H, R_2 = Ac$
4 $R_1 = Me, R_2 = Ac$



5 $R_1 = R_2 = H$
6 Methyl isolimonate $R_1 = Me, R_2 = H$
7 Methyl isolimonate acetate
$R_1 = Me, R_2 = Ac$



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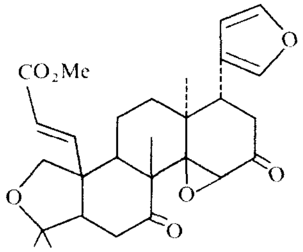

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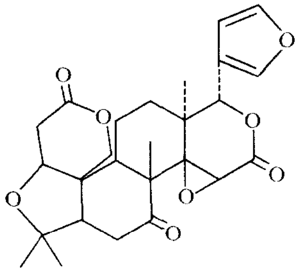

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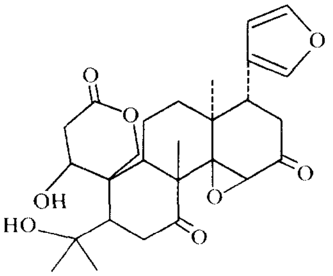

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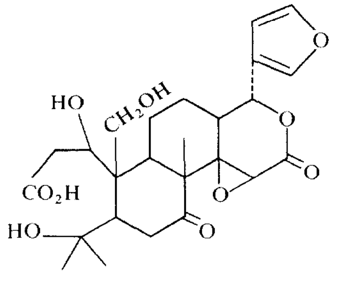

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The neutral fraction contained as major components limonin (**10**), deacetylnomilin, and ichangin (**11**). The latter had previously been isolated by Dreyer from a hybrid of *C. ichangensis* [5], and, as he pointed out, it is a possible precursor of **10**. All of the *C. aurantium* limonoids could be derived biosynthetically from deacetylnomilinic acid (**1**). Lactone ring closure would produce deacetylnomilin, and nomilinic acid (**3**) could be formed by acetylation. Lactone ring closure of **3** would produce nomilin. Hydroxylation of the 19-methyl group of **1** would give the intermediate **12**, which could form **11** by lactone ring closure. It is possible that **12** could have been present in our *C. aurantium* extract, but it would have been converted to **11** under the conditions used for isolation of the limonoids. The 4-hydroxy group of **12** could cyclize with the 19-hydroxyl to produce **5** or with the 1-hydroxyl, followed by lactone ring closure, to give **10**. Alternatively, **10** could be formed from **11** by cyclization.

As mentioned above, **5** is also present in grapefruit seeds, although in very low concentration, and we have found that it is a major constituent of tangerine (*C. reticulata*) seeds. We have also isolated **11** from grapefruit seeds, in which it is one of the minor neutral limonoids.

EXPERIMENTAL

All accurate mass measurements were done under chemical ionization (CI) conditions. NH_3 was the reagent gas and tetraiodoethylene was the standard. Most limonoids do not show a molecular ion in the electron impact mode, but under CI conditions strong M + 1 peaks were observed for all limonoids examined except tertiary alcohols, which gave $MH^+ - 18$ ions. ^{13}C NMR spectral assignments were made on the basis of SFORD spectra, selective heteronuclear decoupling, and comparison with spectra of related limonoids for which assignments had previously been made [3, 7].

Isolation of C. aurantium *limonoids*. Sour orange fruits (Brazilian variety) were obtained from Dr. John Carpenter, USDA Date and Citrus Station, Indio, California. Seeds (900 g fr. wt) were homogenized in 3 l. 0.1 M Tris buffer at pH 8.2 and incubated at 25° for 16 hr. The mixture was filtered through Celite, and the filtrate was acidified to pH 2 and extracted 3 × with CH_2Cl_2. The extracts were combined, washed with H_2O, and evapd to dryness. The residue (1.338 g) was separated into acidic (260 mg) and neutral (900 mg) fractions by the method previously described [1].

The acidic fraction was methylated with CH_2N_2 and chromatographed on a Si gel column, eluted with increasing concentrations (5–15%) of Et_2O in CH_2Cl_2. The first compound eluted was methyl isolimonate (**6**) (60 mg). Although it was homogeneous by TLC in three systems, attempts to crystallize it were unsuccessful, amorphous material being obtained in each case. MS: 503.2243 (M + 1). ($C_{27}H_{35}O_9$ requires: 503.2281). ^{1}H NMR (100 MHz, $CDCl_3$): δ 7.40 (2H, *d*, *J* = 1 Hz, α-furans), 6.32 (1H, *d*, *J* = 1 Hz, β-furan), 5.57 (1H, *s*, H-17), 4.18 (1H, *m*, C-1), 4.00 and 3.93 (2H, 2*d*, *J* = 11 Hz, H-19), 3.93 (1H, *s*, H-15), 3.73 (3H, *s*, Me ester), 1.39, 1.25, 1.18, 1.11 (12H, 4*s*, quarternary Me); ^{13}C NMR (15 MHz, $CDCl_3$): δ 211.8 (7), 173.4 (3), 167.4 (16), 143.0 (α-furan), 141.1 (α-furan), 120.3 (β-furan), 109.7 (β-furan), 83.7 (4), 78.1 (17), 74.9 (1), 69.0 (14), 68.7 (19), 55.3 (15), 52.4 (8), 52.1 (—OMe), 48.7 (10), 48.4 (5), 39.5 (2 or 6), 39.2 (13), 37.2 (2 or 6), 35.8 (9), 28.9 (Me), 28.0 (12), 24.2 (Me), 19.5 (Me), 18.6 (11), 16.8 (Me). Later fractions from the column contained 8 mg of methyl nomilinate (**4**) and 65 mg of methyl deacetylnomilinate (**2**), each identical with authentic samples (TLC and ^{1}H NMR).

Most of the limonin and deacetylnomilin was removed from the neutral fraction by crystallization from CH_2Cl_2–*i*-PrOH. The mother liquor was chromatographed on a Si gel column by the method of Still *et al.* [6], with CH_2Cl_2–Et_2O (70:30) as eluent, to give 84 mg of ichangin (**11**). No authentic sample of **11** was available for comparison, but the mp, IR spectrum, and ^{1}H NMR spectrum of the isolated material, after crystallization from EtOH, were in good agreement with the literature values [5]. ^{13}C NMR (15 MHz, DMSO-d_6): δ 209.3 (7), 171.3 (3), 166.7 (16), 143.2 (α-furan), 141.4 (α-furan), 120.1 (β-furan), 110.1 (β-furan), 77.5 (17), 71.7 (4), 70.3 (1), 67.8 (19), 65.3 (14), 52.1 (15), 52.1 (8), 49.6 (5 or 9), 48.0 (5 or 9), 46.4 (10), 39.0 (2 or 6), 38.2 (2 or 6), 36.4 (13), 32.9 (12), 32.7 (Me), 26.1 (Me), 21.4 (11), 20.6 (Me), 15.8 (Me).

Methyl isolimonate acetate (**7**). MS: 545.2357 (M + 1). ($C_{29}H_{37}O_{10}$ requires: 545.2386). ^{1}H NMR (100 MHz, $CDCl_3$): δ 7.40 (2H, *d*, *J* = 1 Hz, α-furans), 6.32 (1H, *d*, *J* = 1 Hz, β-furan), 5.58 (1H, *s*, H-17), 5.52 (1H, *m*, H-1), 3.94 and 3.61 (2H, 2*d*, *J* = 11 Hz, H-19), 3.91 (1H, *s*, H-15), 3.67 (3H, *s*, Me ester), 2.02 (3H, *s*, acetate Me), 1.39, 1.33, 1.14, 1.14 (12H, 3*s*, quarternary Me).

Methyl anhydroisolimonate (**9**). Compound **7** (5 mg), 0.5 ml MeOH and 0.5 ml of 4 N KOH were heated in a closed tube at 70° for 2.5 hr. The MeOH was removed in vacuum, and the aq. soln was diluted with 2 ml H_2O. After acidification to pH 2 with 3N HCl, the soln was extracted with two 2-ml portions of EtOAc. The extracts were combined, washed with 1 ml H_2O, and evapd to dryness. The residue (4.5 mg) was methylated with CH_2N_2, and the major product was isolated by prep. TLC on Si gel G with cyclohexane–EtOAc (40:60), giving 3 mg of **9**. MS: 485.2220 (M + 1). ($C_{27}H_{33}O_8$ requires: 485.2175). ^{1}H NMR (100 MHz, $CDCl_3$): δ 7.39 (2H, *d*, *J* = 1 Hz, α-furans), 7.10 (1H, *d*, *J* = 16 Hz, H-1), 6.31 (1H, *d*, *J* = 1 Hz, β-furan), 5.87 (1H, *d*, *J* = 16 Hz, H-2), 5.54 (1H, *s*, H-17), 4.04 and 3.88 (2H, 2*d*, *J* = 10 Hz, H-19), 3.94 (1H, *s*, H-15), 3.75 (3H, *s*, Me ester), 1.34, 1.17, 1.15, 1.13, (12H, 4*s*, quarternary Me).

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