Phytochemistry, Vol. 21, No. 9, pp. 2349–2354, 1982.
Printed in Great Britain.

0031-9422/82/092349-06$03.00/0
Pergamon Press Ltd.

7α-OXYGENATED LIMONOIDS FROM THE RUTACEAE

RAYMOND D. BENNETT and SHIN HASEGAWA

USDA, ARS, Fruit and Vegetable Chemistry Laboratory, 263 South Chester Avenue, Pasadena, CA 91106, U.S.A.

(*Revised received* 15 *February* 1982)

Key Word Index—*Citrus paradisi*; *Poncirus trifoliata* × (*Citrus sinensis* × *C. paradisi*); Rutaceae; limonoids; limonol; limonyl acetate; deoxylimonol; 7α-obacunol; 7α-obacunyl acetate; 1(10 → 19) abeo-7α-acetoxy-10β-hydroxyiso-obacunoic acid 3,10-lactone.

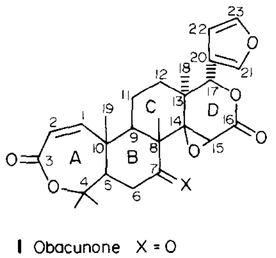
Abstract—Limonol, deoxylimonol, and 7α-obacunol were isolated from *Citrus paradisi* seeds. Limonyl acetate and 7α-obacunyl acetate were isolated from fruit of a *Citrus–Poncirus* hybrid, along with two other new 7α-acetates, 1(10 → 19) abeo-obacun-9(11)-en-7α-yl acetate and 1(10 → 19) abeo-7α-acetoxy-10β-hydroxyiso-obacunoic acid 3,10-lactone. The latter two represent a unique limonoid structural type in which a methyl carbon has been inserted into a ring.

INTRODUCTION

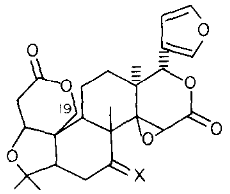
The limonoid bitter principles are found mainly in two plant families, the Rutaceae and the Meliaceae [1]. The latter contains many limonoids with 7α-hydroxy or 7α-acetoxy substituents, but these structural features have thus far been found in only five Rutaceae limonoids, 7α-obacunol from *Casimiroa edulis* [2], 7α-nomilyl acetate from *Dictamnus albus* [3], and tecleanin, 7-deacetylproceranone and 7-deacetylazadirone from *Teclea grandifolia* [4]. Here we report the isolation of three 7α-hydroxylimonoids from grapefruit (*Citrus paradisi*) seeds and four 7α-acetoxylimonoids from fruit of a *Citrus–Poncirus* hybrid.

RESULTS AND DISCUSSION

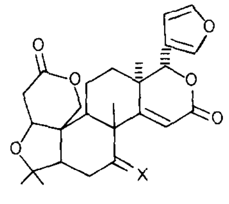
Three minor limonoids were isolated from a grapefruit seed extract by chromatography and crystallization. Two of them were identified as known limonoids, 7α-obacunol (**2**), which as mentioned above had previously been isolated from another Rutaceae species, and limonol (**5**), which was not previously known as a natural product but had been prepared synthetically [5]. The ^{1}H NMR spectrum of the third isolated compound, a new limonoid, showed four quaternary methyl signals, indicating that it was related to limonin (**4**) in which C-19 is oxygenated, rather than to compounds such as obacunone (**1**) which contain five quaternary methyl groups. Three



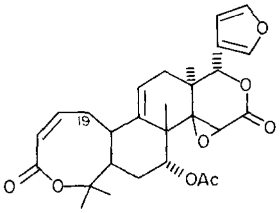
1 Obacunone X = O
2 7α-Obacunol X = α-OH, β-H
3 7α-Obacunyl acetate X = α-OAc, β-H



4 Limonin X = O
5 Limonol X = α-OH, β-H
6 Limonyl acetate X = α-OAc, β-H



7 Deoxylimonin X = O
8 Deoxylimonol X = α-OH, β-H



9 1-(10→19) Abeo-obacun-9(11)-en-7α-yl acetate features of the spectrum suggested that the compound was related to deoxylimonin (**7**): the downfield position of the H-15 signal, identifiable by its strong ringing; the upfield position of the H-17 resonance; and the complexity of the α-furan splitting pattern[6]. The lack of a ketone carbonyl resonance in the ^{13}C NMR spectrum suggested the presence of a 7-hydroxyl group, rather than the 7-ketone found in most citrus limonoids. A signal at *ca* δ4.15 in the ^{1}H NMR spectrum, which could be attributed to the H-7 resonance, was partly overlapped with another resonance, but it appeared to be relatively narrow in width. The H-7 resonance in **5** is a broadened singlet, whereas in epilimonol (7β-OH) it is a complex multiplet. Thus, the NMR evidence suggested that the new compound was deoxylimonol (**8**). This was confirmed when **8** was synthesized by reduction of **5** with chromium dichloride. The synthetic product was identical with the compound isolated from grapefruit seeds.

During a survey of the limonoid contents of various citrus species and hybrids by TLC, we found that a hybrid, *Poncirus trifoliata* × (*C. sinensis* × *C. paradisi*) contained, along with limonin and nomilin, four Ehrlich-positive components different in mobility from the common citrus limonoids. Although this hybrid had relatively few seeds, the fruit tissue contained much higher concentrations of limonoids than are usually found in citrus fruits. Therefore, whole fruits were used for extraction and isolation of the new limonoids. Two of these compounds proved to be limonyl acetate (**6**) and 7α-obacunyl acetate (**3**). Limonyl acetate (**6**) was not previously known to be a natural product, while **3** has been isolated from *Cneorum tricoccin* (Cneoraceae)[7].

Two other limonoids were isolated which appeared to be 7α-acetates from their ^{1}H NMR spectra. However, the spectra also indicated that they represented a unique structural type of limonoid. Compound I (structure **9**) contained only four quaternary methyl groups, but the other methyl group had not been oxygenated as in compounds such as **4**. Instead, the SFORD ^{13}C NMR spectrum showed that compound I had an extra methylene group attached to two other carbon atoms. This suggested that a methyl carbon had been inserted into one of the rings. The positions of the furan, H-17, and H-15 resonances indicated a normal D-ring lactone structure. A broadened singlet near δ4.8 and a methyl resonance near δ2.0 were consistent with the presence of a 7α-acetate group, as was the position of the H-15 resonance, which is strongly influenced by the nature of the C-7 functionality[6]. The remaining identifiable features of the ^{1}H NMR spectrum were three olefinic resonances, referred to hereafter as A, B, and C. After treatment with the shift reagent Eu(FOD)$_3$ to spread out the spectrum, five other previously overlapped signals (D, E, F, G, H) could be observed and decoupling experiments could be carried out. The results of these experiments are shown in Table 1. Protons A, B, D, E, and F were thereby shown to be included in the partial structure **A** (Scheme 1). The expected coupling between E and F could not be demonstrated, because resonances were broadened in the presence of Eu(FOD)$_3$ to the extent that small couplings were difficult to detect, but the structure is still unambiguous because of the demonstrated coupling of F to D, which is a geminal partner of E. The carbon atom bearing proton B must be adjacent to either a qua-

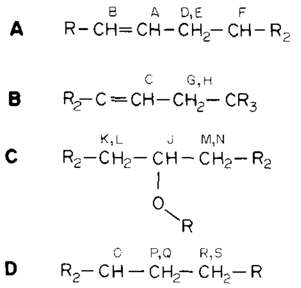
Table 1. Homonuclear decoupling experiments on protons in Eu(FOD)$_3$-shifted ^{1}H NMR spectra of compounds I* and II†

Signal irradiated	Chemical shift	Multiplicity (*J*, Hz)	Signals affected by irradiation
A	6.2	*m*	B, D, E
B	6.0	*d*(12)	A
C	5.7	*dd*(2, 7)	G, H
D	2.9	*m*	A, E, F
E	2.1	*m*	A, D
F	3.0	*m*	—
G	2.1	*m*	C, H
H	1.8	*m*	C, G
J	4.4	*m*	L, M, N
K	2.8	*d*(19)	J, L
L	2.6	*dd*(5, 19)	K
M	2.1	*d*(13)	N
N	1.7	*dd*(3, 13)	M
O	2.4	*dd*(7, 12)	—
P	1.7	*m*	O, Q
Q	1.5	*m*	O, P
R	1.3	*m*	P, Q
S	1.3	*m*	P, Q

Spectra were run in CDCl$_3$ at 100 MHz. Chemical shifts (δ) are the approximate positions of the resonances in the original spectra prior to treatment with Eu(FOD)$_3$.

*Signals A–H.

†Signals J–S.



Scheme 1. Partial structures of compounds **1** and **2**.

ternary carbon or a carbonyl group, since B is coupled only to A. The ^{13}C NMR spectrum showed three carbonyl resonances, one of which may be assigned to the D-ring lactone and one to the 7α-acetate, whose presence was shown by the proton spectrum. The chemical shift of the other carbonyl (δ167) indicated that it is a lactone carbonyl, and the downfield position of two of the quaternary methyl signals in the proton spectrum, at δ1.59 and 1.42, suggested that C-4 is involved in the lactone linkage. The two C-4 methyl groups are normally found upfield of δ1.4, unless, as in compounds such as **1**, they are part of a lactone ring. A strong UV absorbance at 210 nm was indicative of the presence of an α,β-unsaturated lactone, as in compound **1**. Therefore, the double bond represented by protons A and B is assigned to the 1,2-position. Protons D and E would then be the methylene group derived by insertion of C-19 into the A-ring of an obacunone-type precursor, and proton F would be H-10.

The coupling relationships between protons C, G, and H shown in Table 1, together with the fact that G and H showed no evidence of coupling to any other protons, lead to the partial structure **B** (Scheme 1). This grouping can only be accommodated in the C-ring, with proton C located at the 11-position and protons G and H attached to C-12. This assignment leads to the structure **9** for compound I. The proposed structure is consistent with the multiplicities shown for the individual carbon signals in the SFORD ^{13}C NMR spectrum and with the empirical formula given by accurate mass measurement.

The ^{1}H NMR spectrum of compound II (structure **10**) was similar in several respects to that of compound **9**. The furan, H-17, and H-15 resonances were consistent with a normal limonoid D-ring lactone. A broadened singlet near δ4.5 was similar in appearance and chemical shift to the resonance assigned to H-7 in compound **9**, and the chemical shifts of H-15 and a methyl singlet near δ2.1 also indicated the presence of a 7α-acetate group. Like compound **9**, compound II showed only four quaternary methyl signals, and the absence of any oxymethylene resonances suggested that as in **9** a methyl group had been inserted into a ring. The proton spectrum differed from that of compound **9** in that: (a) it lacked any olefinic resonances; and (b) it contained a one-proton multiplet (J) near δ4.4. The position of this resonance indicated the presence of an oxymethine carbon, but the substituent did not appear to be a hydroxyl group. Upon treatment with acetic anhydride and pyridine compound II was recovered unchanged. Also, no hydroxyl protons were observed in the proton spectrum, even in the presence of DMSO-d_6, and the IR spectrum showed no hydroxyl bands. Thus, the carbon atom bearing proton J appeared to be part of a cyclic ether or lactone ring.

Upon treatment with Eu(FOD)$_3$ to spread out the proton spectrum, two resonances, K and L, moved downfield much faster than the others. When J was irradiated, L collapsed to a doublet and formed an AB system ($J = 19$ Hz) with K. If K and L were vicinal protons, both carbons would have to be methine. However, the SFORD ^{13}C NMR spectrum showed only two methine resonances upfield of the oxygen-bearing ones, and both were shown by selective heteronuclear decoupling to be coupled to protons other than K or L. Therefore, K and L must be attached to the same carbon atom, and the coupling between J and K must have been too small to be observed. The chemical shifts of K and L indicated that they are next to a carbonyl group, as did the strong effect of Eu(FOD)$_3$ upon them. The ^{13}C NMR spectrum showed three carbonyl resonances, two of which can be assigned to the D-ring lactone and the 7-acetate. The chemical shift of the third one indicated that it was a lactone carbonyl, presumably adjacent to K and L.

Proton J was also coupled to two others, M and N (Table 1), which were shown to be a geminal pair by the same means used for K and L above. Thus, proton J is on a carbon bounded by two methylene groups (C, Scheme 1). Such a structure could rationally be derived from compound **9** by 1,4-addition of a hydroxyl group to the A-ring double bond. Protons K and L would then be on C-2, J on C-1, and M and N on C-19.

Three other protons, O, P, and Q, were found to be mutually coupled (Table 1); O was shown to be a methine proton by selective heteronuclear decoupling and therefore P and Q must represent a geminal pair. Both P and Q were each also coupled to two protons, R and S. The latter two were not separated sufficiently for homonuclear decoupling, but they were shown to be a geminal pair by selective heteronuclear decoupling. Thus, these decoupling experiments demonstrate the presence of the partial structure **D** (Scheme 1). This indicates that compound II contains a normal limonoid C-ring, with O attached to C-9, P and Q to C-11, and R and S to C-12.

The following structural features have thus far been shown to be present in compound II: furan ring, normal limonoid C- and D-rings, 7α-acetate, C-1 oxymethine, C-2 and C-19 methylenes, and a C-3 lactone carbonyl. Those carbons remaining to be assigned in the SFORD ^{13}C NMR spectrum (aside from quaternary methyls) include two quaternary oxycarbons at δ73 and 80, a methine carbon at δ45, and a methylene group at δ24. Since there are no free hydroxyl groups in the molecule, one of the quaternary oxycarbons must represent the terminus of the lactone and the other one must form a cyclic ether with the C-1 oxymethine. One of the quaternary oxycarbons can be assigned to C-4, which is invariably oxygenated in Rutaceae limonoids, and the methylene group to C-6, since the appearance and chemical shift of the H-7 resonance in the proton spectrum are consistent with those of other 7α-

Table 2. ^1H NMR spectra of 7α-oxygenated limonoids

Proton	**3**	**8**	**9**	**10**	**11**
α-Furans	7.40(*t*, 1)	7.46(*d*, 1) 7.43(*d*, 1)	7.40(*t*, 1)	7.40(*t*, 1)	7.40(*t*, 1)
β-Furan	6.32(*d*, 1)	6.41(*d*, 1)	6.34(*d*, 1)	6.32(*d*, 1)	6.33(*d*, 1)
H-17	5.58	4.98	5.66	5.63	5.62
H-19	—	4.46	—	—	—
H-11	—	—	5.70(*dd*, 2, 7)	—	—
H-7	4.49	4.14	4.75	4.53	3.56
H-1	6.43(*d*, 12)	4.06(*m*)	6.22(*m*)	4.40(*m*)	4.40(*m*)
H-2	5.87(*d*, 12)		6.02(*d*, 12)		
H-15	3.49	5.94	3.61	3.53	3.92
C-Methyls	1.42	1.28	1.55	1.39	1.33
	1.34	1.18	1.42	1.18	1.25
	1.31	1.13	1.02	1.18	1.17
	1.22	1.10	1.02	1.15	1.17
	1.11				
Acetate methyl	2.11	—	2.03	2.13	—

Chemical shifts are in δ units; signal multiplicities (unless singlets) and coupling constants (in Hz) are in parentheses. Samples were run in $CDCl_3$ at 100 MHz.

Table 3. ^{13}C NMR spectra of 7α-oxygenated limonoids

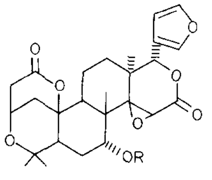
Carbon no.	**2**	**3**	**5**	**6**	**8**	**9**	**10**
1	157.3	155.8	80.9	80.6	80.1	139.2	65.0
2	120.5	121.0	36.8	36.6	36.4	124.6	38.2
3	168.6	167.3*	171.5	170.6*	171.1	167.5*	169.8
4	85.5	84.6	81.2	81.0	81.0	84.3	80.8
5	47.9	49.2	52.9	53.9	52.2	41.1*	45.3
6	30.6	26.9*	27.5*	24.2	26.5	30.5*	24.9
7	69.0	72.9	70.8	74.3	72.1	74.8	72.5
8	43.9*	42.1	44.8*	43.8*	46.8	41.8	41.4
9	39.9	41.4	42.9	43.9	39.8	144.8	39.7
10	43.1*	43.9	46.8*	46.5*	46.8	33.7*	73.0
11	16.4	16.4	17.9	17.7	17.3	122.1	14.4
12	26.8	26.3*	27.1*	26.8	26.5	23.8	24.6
13	38.0	38.5	39.1	39.4	38.7	39.7	39.0
14	70.1	69.7	70.3	70.0	175.9	67.9	69.8
15	57.2	56.5	58.3	56.9	114.5	53.3	57.3
16	167,9	167.2*	169.0	168.3	166.1	167.2*	167.5
17	78.6	78.3	79.3	79.2	82.5	77.6	78.1
19	—	—	67.1	66.5	66.5	29.2*	33.9
20	120.5	120.4	122.1	121.7	121.5	120.3	120.4
21	141.1	141.3	142.6	142.6	142.4	142.0	141.3
22	109.9	109.9	111.2	111.0	111.0	109.9	109.9
23	142.9	143.1	144.2	144.2	144.0	143.1	143.1
C-Methyls	32.0	32.1	30.7	30.5	30.7	40.5	32.6
	26.8	26.5	22.0	21.8	26.3	28.3	24.6
	18.5	18.2	18.9	18.4	22.0	23.1	18.7
	18.1	18.1	17.9	18.1	17.9	15.7	16.8
	16.0	16.0	—	—	—	—	—
Acetate carbonyl	—	169.9	—	170.9*	—	169.5	169.8
Acetate methyl	—	21.1	—	21.4	—	21.1	21.0

Chemical shifts are in δ units. Compounds **2**, **3**, **9**, and **10** were run in $CDCl_3$, and compounds **5**, **6**, and **8** in CD_3CN, all at 15 MHz.

*Assignments in the same vertical column may be reversed.

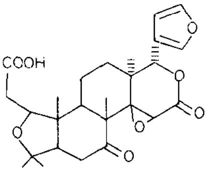
acetates in which C-6 is methylene. This leaves C-5 and C-10 as possible sites of the remaining quaternary oxycarbon and the methine carbon. A methine proton at C-5 would be coupled to the C-6 protons, but in the Eu(FOD)$_3$-shifted spectrum these three proton signals were not separated, so decoupling experiments were not possible. However, when compound 11 was treated with base to hydrolyse the 7α-acetoxy group and then acidified to close the lactone rings, the resulting 7α-alcohol complexed strongly with Eu(FOD)$_3$, and the methine and methylene resonances were then separated in the proton spectrum. Decoupling experiments showed that they were indeed coupled to each other, and therefore the methine is located at C-5. This establishes C-10 as the site of the other quaternary oxycarbon.

Two possible alternative structures still remained: a 3,4-lactone and 1,10-cyclic ether, or a 3,10-lactone and 1,4-cyclic ether. In the former the ether would be a four-membered ring, which is inconsistent with the coupling constants (1 and 3 Hz) observed between H-1 and the two H-19 protons. Vicinal coupling constants for oxetanes range between 5 and 11 Hz[8]. Also none of the quaternary methyl resonances in the ^{1}H NMR spectrum was located above δ1.4, which indicates that C-4 is not part of a lactone ring. With a 3,10-lactone and 1,4-cyclic ether, both rings would be six-membered. A Dreiding model showed that the ether ring must be in the chair conformation or formation of the lactone ring is not possible. With the latter in the chair conformation H-1 forms a *ca* 60° angle with each of the four H-2 and H-19 protons. In such a structure the coupling constants between H-1 and these other protons would all be small, as is in fact observed in the ^{1}H NMR spectrum of compound 11 ($J = 5$, 3, 1 and < 1 Hz). Therefore, this compound is assigned the structure **10**.



10 1-(10→19)Abeo-7α-acetoxy-10β-hydroxyiso-obacunoic acid 3,10-lactone R = Ac

11 1-(10→19)Abeo-7α, 10β-dihydroxy iso-obacunoic acid 3,10-lactone R = H



12 Iso-obacunoic acid

One remaining question concerned the configuration of the C-10 oxygen. Formation of the lactone and ether rings is equally possible with the oxygen either α- or β-orientated, and both configurations are consistent with the observed coupling constants However, when the H-1 resonance was irradiated, one of the H-19 doublets was considerably broader than the other, and likewise one of the H-2 doublets was broader than the other. This suggested the presence of a long-range H-2, H-19 coupling, which was confirmed by a decoupling experiment. Dreiding models show that in both configurations for the C-10 oxygen one H-2 and one H-19 proton are in the *W*-conformation necessary for long-range coupling. However, with a 10α-oxygen substituent the other H-19 proton also forms a *W*-conformation with H-5; such a conformation is not present with a 10β-oxygen orientation. Since the other H-19 resonance appeared to be so narrow as to exclude long-range coupling, the 10β-oxygen configuration is preferred.

Compound **9** is structurally related to obacunone (**1**), and so we have assigned it as a derivative of the latter, in accord with the recent IUPAC recommendations on nomenclature of new natural products[9]. It is therefore named 1(10→19) abeo-obacun-9(11)en-7α-yl acetate. Likewise, compound **10** is named as a derivative of iso-obacunoic acid (**12**) and is 1(10→19) abeo-7α-acetoxy-10β-hydroxyiso-obacunoic acid 3,10-lactone.

It seems most likely that compound **9** is a biosynthetic precursor of **10**, although the reverse possibility cannot be excluded. The 1,4-cyclic ether system of compound **10** could be formed by opening the lactone ring of **9** followed by addition of the 4-hydroxyl group to the 1,2-double bond, analogous to the mechanism for the conversion of **1** to **12**[1]. A shift of the 9,11-double bond to the 9,10-position, followed by addition of the 3-carboxyl group at C-10 would then produce compound **10**. The finding of seven 7α-oxygenated limonoids in Rutaceae species as reported here, along with the three previous reports cited above, suggests that the occurrence of this structural feature may be more common among limonoids of this family than was previously recognized.

EXPERIMENTAL

Mps are uncorr. Si gel G plates were used for TLC. 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 M$^+$ in the electron impact mode, but under CI conditions strong $[M+1]^+$ peaks are obtained for all limonoids except tertiary alcohols. ^{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[10, 11].

Isolation of C. paradisi *limonoids*. 3 kg dried grapefruit seeds were ground and defatted by extraction with hexane. The seeds were then extracted continuously with boiling Me$_2$CO overnight. The Me$_2$CO extract was evaporated to dryness. The residue was taken up in CHCl$_3$ and washed twice with H$_2$O. The CHCl$_3$ was evaporated and most of the limonin was removed from the residue by crystallization from CH$_2$Cl$_2$–*iso*-PrOH. The combined mother liquors were evaporated to dryness and chromatographed on a Si gel column eluted with increasing concns of MeOH in CH$_2$Cl$_2$. Fractions enriched in obacunone, limonin, and deacetylnomilin, respectively, were thus obtained.

The obacunone fraction was rechromatographed on a Si gel column eluted with increasing concns of EtOAc in CHCl$_3$. Fractions containing mainly obacunone and a slightly more polar limonoid were pooled and most of the obacunone was removed by crystallization from MeOH. The mother liquor was chromatographed on a Si gel column eluted with CHCl$_3$–MeOH (99:1). The other limonoid (26 mg) was obtained chromatographically pure, and it was identical (TLC and ^{1}H NMR) with a synthesized sample[2, 12] of 7α-obacunol (**2**).

The limonin fraction from the first column was rechromatographed on a Si gel column eluted with increasing concns of EtOAc in $CHCl_3$. Fractions containing mainly limonin and a slightly more polar limonoid were combined and most of the limonin was removed by crystallization from CH_2Cl_2–*iso*-PrOH. The mother liquor was chromatographed on a Si gel column eluted with CH_2Cl_2–Et_2O (90:10). The other limonoid was obtained chromatographically pure (68 mg), and it was identical (TLC, ^1H NMR) with a synthesized sample[13] of limonol.

The deacetylnomilin fraction from the first column was crystallized from EtOAc to remove most of this limonoid. The mother liquor, which contained mainly ichangin[14] and a new limonoid, was chromatographed on a Si gel column eluted with increasing amounts of MeOH in CH_2Cl_2. The new limonoid (23 mg) was crystallized from CH_2Cl–Et_2O; mp 250–252°. It was identical (TLC, ^1H NMR) with a sample of deoxylimonol (**8**) prepared by synthesis (see below). MS:457.2210 $[M+1]^+$. ($C_{26}H_{33}O_7$ requires: 457.2226.)

Isolation of Citrus–Poncirus *hybrid limonoids.* A tree in the citrus groves of the Citrus Research Center, University of California at Riverside, was identified as a hybrid, *P. trifoliata* × (*C. sinensis* × *C. paradisi*), by Mr. Paul Moore. Fresh fruit (1 kg) from this tree was homogenized with Me_2CO. The homogenate was filtered and the Me_2CO was removed from the filtrate *in vacuo*. The aq. residue was extracted twice with CH_2Cl_2. The extracts were combined, washed with H_2O, and evaporated to dryness. The residue was chromatographed on a Si gel column eluted with increasing concns of EtOAc in $CHCl_3$. The first fraction contained a limonoid, along with some flavonoid impurities. This fraction was rechromatographed on a Si gel column eluted with $CHCl_3$–MeOH (99:1). The limonoid fraction was washed with hexane to remove oily impurities. The compound thus obtained (15 mg) was identical (TLC, ^1H NMR) with a sample of 7α-obacunyl acetate (**3**) prepared by acetylation of 7α-obacunol. MS: 499.2338 $[M+1]^+$. ($C_{28}H_{35}O_8$ requires: 499.2331.)

The next limonoid eluted from the first column was washed with hexane to remove some oily impurities and then purified by prep. TLC on Si gel G with CH_2Cl_2–Et_2O (90:10) to give 5 mg 1-(10→19) abeo-obacun-9(11) en-7α-yl acetate (**9**). MS: 497.2164 $[M+1]^+$. ($C_{28}H_{33}O_8$ requires: 497.2175.)

The next fraction eluted from the first column contained two limonoids. After washing with hexane to remove oily impurities the two were separated by chromatography on a Si gel column eluted with $CHCl_3$–MeOH (99:1). The first limonoid, weighing 25 mg, was 1-(10→19) abeo-7α-acetoxy-10β-hydroxyiso-obacunoic acid 3,10-lactone (**10**). After crystallization from MeOH it had mp 271–274°. MS: 515.2265 $[M+1]^+$. ($C_{28}H_{35}O_9$ requires: 515.2281.)

The second limonoid from the last column (5 mg) was identical (TLC and ^1H NMR) with a synthesized sample[13] of limonyl acetate (**6**).

The last two fractions from the first Si gel column contained 82 mg limonin and 56 mg nomilin, respectively, both identical (TLC and ^1H NMR) with authentic samples.

1-(10→19)*abeo-7α,10β-dihydroxyiso-obacunoic acid* 3,10-*lactone* (**11**). To a soln of 4 mg compound **10** in 1 ml MeOH was added 20 μl 5 N KOH soln. After 16 hr at 25° 1 ml H_2O was added and the soln was concd to 1 ml under N_2, acidified to pH 3 with 1 N HCl, and extracted twice with 1 ml portions of EtOAc. The extracts were combined, washed with H_2O, and evaporated to yield 3.5 mg **11**. MS: 473.2194 $[M+1]^+$. ($C_{26}H_{33}O_8$ requires: 473.2175.)

Synthesis of deoxylimonol (**8**). To a soln of 87 mg limonol in 6 ml MeCN in a screw-capped test tube was added 0.5 ml HOAc and enough $CrCl_2$ soln (Fisher Scientific) to completely fill the tube (*ca* 2.5 ml). The tube was then tightly capped and kept at 25° for 3 days. The reaction mixture was poured into 200 ml H_2O, concd to 150 ml *in vacuo*, and extracted with two 50 ml portions of CH_2Cl_2. The extracts were combined, washed with H_2O, and evaporated to dryness. The product **8** (81 mg) was >90% pure by TLC and was identical with the compound isolated from *C. paradisi*.

Acknowledgements—We thank V. P. Maier and R. M. Horowitz for helpful discussions and S. M. Poling for running the mass spectra. Reference to a product name or company does not imply endorsement of that product or company by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

REFERENCES

1. Dreyer, D. L. (1968) *Fortschr. Chem. Org. Naturstoffe* **26**, 190.
2. Dreyer, D. L. (1968) *J. Org. Chem.* **33**, 3577.
3. Monkovic, I., Spenser, I. D. and Plunkett, A. O. (1967) *Can. J. Chem.* **45**, 1935.
4. Ayafor, J. F., Sondengam, B. L., Connolly, J. D., Rycroft, D. S. and Okogun, J. I. (1981), *J. Chem. Soc. Perkin Trans* 1, 1750.
5. Chandler, B. V. and Kefford, J. F. (1951) *Aust. J. Sci.* **14**, 24.
6. Dreyer, D. L. (1965) *Tetrahedron* **21**, 75.
7. Straka, H., Albers, F. and Mondon, A. (1976) *Beitr. Biol. Pflanz.* **52**, 267.
8. Balsamo, O., Ceccarelli, G., Crotti, P. and Macchia, F. (1975) *J. Org. Chem.* **40**, 473.
9. Anonymous (1978) *Eur. J. Biochem.* **86**, 1.
10. Dreyer, D. L., Bennett, R. D. and Basa, S. C. (1976) *Tetrahedron* **32**, 2367.
11. Bennett, R. D. and Hasegawa, S. (1981) *Tetrahedron* **37**, 17.
12. Dean, F. M. and Geissman, T. A. (1958) *J. Org. Chem.* **23**, 596.
13. Melera, A., Schaffner, K., Arigoni, D. and Jeger, O. (1957) *Helv. Chim. Acta* **40**, 1420.
14. Dreyer, D. L. (1966) *J. Org. Chem.* **31**, 2279.