Bitter Limonoids from the Fruit of *Melia azedarach* L. *var. japonica* Makino[1)]

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Two new bitter limonoids, ohchinolal (**1**) and ohchinin (**2**), have been isolated from the fruit of *Melia azedarach* L. *var. japonica* Makino, and their structures have been established as **1** and **2** by spectroscopic and chemical methods. The complete assignments of the ^1H and ^{13}C NMR data are described.

Recent investigations of the plants of the family Meliaceae have resulted in the isolation of many limonoids of biological interest, such as those with potent cytotoxic activity[2)] and with insect antifeedant and insecticidal properties.[3,4)] Our continuing search for biologically active constituents of *Melia azedarach* L. *var. japonica* Makino has now led to the isolation of two new bitter limonoids, designated as ohchinolal and ohchinin, the latter as the natural precursor of ohchinin acetate (**3**).[5)] The present paper deals with the structural determination of these new compounds.

Ohchinolal (**1**), $C_{34}H_{44}O_{10}$, mp 163—164.5 °C, $[\alpha]_D^{23}$

TABLE 1. ^1H NMR SPECTRA AT 400 MHz OF OHCHINOLAL (**1**) AND OHCHININ (**2**)[a)]

		1		**2**
H_1		5.08 (dd, 3.2, 2.7)		5.09 (dd, 2.7, 2.7)
$H_{2\alpha}$		2.22 (ddd, 16.2, 3.5, 2.7)		2.31 (ddd, 13.8, 3.0, 2.7)
$H_{2\beta}$		2.02 (ddd, 16.2, 3.2, 2.7)		2.14 (ddd, 13.8, 3.0, 2.7)
H_3		3.75 (ddd, 9.2, 3.5, 2.7)		3.91 (ddd, 8.9, 3.0, 3.0)
H_5		3.66 (d, 12.2)		2.78 (d, 12.4)
H_6		5.26 (dd, 12.2, 2.7)		4.04 (dd, 12.4, 3.2)
H_7		4.03 (d, 2.7)		4.21 (d, 3.2)
H_9		2.84 (dd, 8.4, 3.2)		2.71 (dd, 9.5, 3.0)
H_{11}		2.24 (dd, 15.7,3.2)		2.19 (dd, 15.1, 3.0)
		2.32 (dd, 15.7, 8.4)		2.34 (dd, 15.1, 9.5)
H_{15}		5.49 (dddq, 7.8, 6.8, 1.8, 1.4)		5.48 (dddq, 8.4, 6.8, 1.9, 1.4)
$H_{16\alpha}$		2.27 (dd, 12.4, 6.8)		2.15 (dd, 12.2, 8.4)
$H_{16\beta}$		2.09 (ddd, 12.4, 9.2, 7.8)		2.24 (ddd, 12.2, 8.6, 6.8)
H_{17}		3.65 (dd, 9.2, 1.8)		3.61 (dd, 8.6, 1.9)
H_{21}		7.25 (dd, 1.9, 0.8)		7.14 (dd, 1.4, 1.0)
H_{22}		6.29 (dd, 1.6, 0.8)		6.18 (dd, 1.9, 1.0)
H_{23}		7.33 (dd, 1.9, 1.6)		7.13 (dd, 1.9, 1.4)
H_{28}		9.74 (s)		3.66 (d, 7.6)
				4.17 (d, 7.6)
Me_{18}		1.65 (d, 1.4)		1.65 (d, 1.4)
Me_{19}		1.08 (s)		0.98 (s)
Me_{29}		1.00 (s)		1.17 (s)
Me_{30}		1.41 (s)		1.32 (s)
CO_2Me		3.23 (s)		3.11 (s)
OAc		1.98 (s)		
OH		2.73 (d, 9.2)[b)]		2.46 (d, 8.9)[b)]
OTig[c)]	$H_{3'}$	6.94 (qq, 7.0, 1.6)		
	$Me_{4'}$	1.86 (dq, 7.0, 1.1)		
	$Me_{5'}$	1.94 (dq, 1.6, 1.1)		
Ocin[c)]			$H_{2'}$	6.50 (d, 15.9)
			$H_{3'}$	7.72 (d, 15.9)
			$H_{5'}$	7.57 (m)
			$H_{6'}$	7.41 (m)
			$H_{7'}$	7.41 (m)

a) The spectra were determined at 25 °C in $CDCl_3$ solutions, with TMS as the internal standard. The multiplicity and J values (in Hz) are in parentheses. b) These signals disappeared on addition of D_2O.

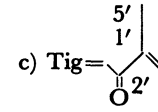
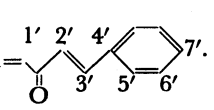
c) Tig=  ; Cin=  .

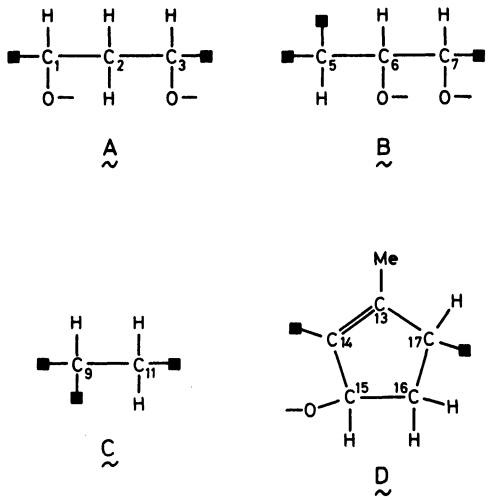
TABLE 2. ^{13}C CHEMICAL SHIFTS OF OHCHINOLAL (**1**) AND OHCHININ (**2**)[a)]

Carbon	1	2	Carbon	1	2
1	75.2 d	73.4 d	19	16.9 q	17.0 q
2	28.5 t	30.7 t	20	127.2 s	127.3 s
3	76.5 d	72.7 d	21	139.1 d	138.9 d
4	47.2 s	44.4 s	22	110.8 d	110.8 d
5	35.2 d	39.7 d	23	143.1 d	143.0 d
6	69.1 d	71.1 d	28	206.9 d	78.1 t
7	86 2 d	86.3 d	29	14.0 q	20.0 q
8	49.2 s	49.2 s	30	17.1 q	13.2 q
9	39.7 d	39.1 d	OCH_3	51.5 q	51.6 q
10	42.3 s	41.2 s	$OCO\underline{C}H_3$	20.8 q	
11	30.4 t	30.8 t	$O\underline{C}OCH_3$	170.4 s	
12	172.6 s	172.8 s	1′	166.4 s	165.7 s
13	135.7 s	134.6 s	2′	128.7 s	118.3 d
14	146.4 s	146.7 s	3′	143.1 d	145.6 d
15	87.7 d	88.2 d	4′	12.1 q	135.1 s
16	41.3 t	41.3 t	5′	14.5 q	128.4 d
17	49.8 d	49.7 d	6′		129.2 d
18	13.0 q	15.4 q	7′		130.7 d

a) The spectra were measured at 100.61 MHz in $CDCl_3$ solutions, and the shifts are given in ppm (δ) relative to the internal TMS. Assignments were made by means of off-resonance and selective proton-noise decoupling techniques.

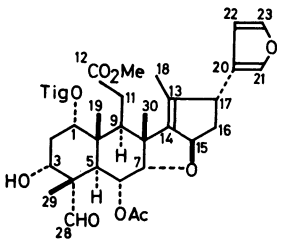
$+52°$ (c 0.22, EtOH), was isolated from the methanol extract of the fruit as colorless prisms in a 0.013% yield by means of extensive column chromatography. It had IR absorptions attributable to a hydroxyl group (3475 cm^{-1}) and a β-substituted furan ring (3150, 1500, and 875 cm^{-1}). The 400 MHz ^1H NMR spectrum showed signals due to three tertiary methyl groups at $\delta=1.00$, 1.08, and 1.41, one vinylic methyl group at $\delta=1.65$ (d, $J=1.4$ Hz), one acetyl group at $\delta=1.98$, one secondary hydroxyl group at $\delta=2.73$ (d, $J=9.2$ Hz), one ester methyl group at $\delta=3.23$, and one formyl group at $\delta=9.74$ (s). The presence of a tigloyl group was also evident from the ^1H NMR signals at $\delta=1.86$ (3H, dq, $J=7.0$ and 1.1 Hz), 1.94 (3H, dq, $J=1.6$ and 1.1 Hz), and 6.94 (1H, qq, $J=7.0$ and 1.6 Hz), and the MS fragment at m/z 529 [$M^+-CH_3CH=C(CH_3)-C\equiv O^+$]. In addition, extensive ^1H NMDR studies, as summarized in Table 1, revealed the presence of four partial structures (**A**), (**B**), (**C**), and (**D**), the last one showing the following long-range couplings: $J_{H_{15},\,H_{17}}=1.8$ Hz, $J_{H_{15},\,H_{18}}=1.4$ Hz, and $J_{H_{17},\,H_{18}}\approx 0.1$ Hz. The ^{13}C NMR data for **1**, obtained with the assistance of off-resonance and selective proton-noise decoupling techniques, showed the presence of another ester-type carbonyl carbon atom at δ_C 172.6, along with three methylene groups, three methine groups, five oxygen-bearing methine groups, three quaternary carbon atoms, and one tetrasubstituted double bond.

The spectral data and the analogy with the congeners isolated from Melia species[5,6] suggested a close structural relationship between **1** and salannin (**4**).[7] The lack of the ^{13}C NMR signal characteristic for the C_{28} of salannin-type limonoids around δ_C 78 and the paramagnetic shift of the ^1H NMR signal due to H_6 indicate the absence of the C_6–O–C_{28} ether linkage which exists in **4**. The location of the formyl group at C_{28} was revealed by the observation of a nuclear Overhauser effect (NOE) on H_5 (13%) upon the irradiation of the



formyl proton. The positions of the secondary hydroxyl and tigloyloxyl groups were elucidated as follows. The oxidation of **1** with pyridinium chlorochromate, accompanied by the elimination of tiglic acid, gave an enone (**5**), $C_{29}H_{34}O_8$, as an oil. The ^1H NMR spectrum of **5** contained a typical AB quartet at $\delta=5.99$ and 6.99 ($J=10.3$ Hz, H_2 and H_1 respectively) indicative of an α,β-unsaturated ketone, while the irradiation of the methylene protons at C_{11} under NOE conditions produced a clear enhancement of the intensity of the latter signal (5.2%). This fact suggested the presence of a $-C_1(H)=C_2(H)-C_3(=O)-$ grouping in **5** and, hence, the location of the tigloyloxyl group at C_1 and the secondary hydroxyl group at C_3 in **1**. The appearance of the signal of H_6 in **1** at a lower field ($\delta=5.26$) as compared with that of **3** ($\delta=4.05$)[5] revealed the position of the remaining acetoxyl group at C_6.

The ^1H NMDR studies of **1** for the partial structure (**D**) elucidated the chemical shift and the coupling



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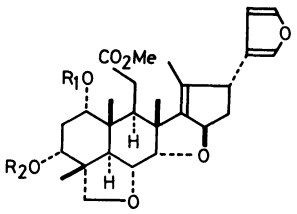
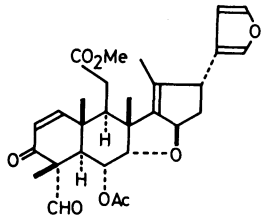


2 R_1 = Cin, R_2 = H
3 R_1 = Cin, R_2 = Ac
4 R_1 = Tig, R_2 = Ac



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constant values, as summarized in Table 1. From the examination of the J values and the model, it is concluded that H_{15} and H_{17} face opposite sides. The α-configuration for C_{17}-furan is common to all the limonoids;[8] consequently, the C_{15}-ether linkage of **1** must be β-oriented. The α-configuration of the C–O linkages at C_1, C_3, C_6, and C_7 was evident from the J values of their α-protons.[3a,5] From the evidence outlined above, we proposed Structure **1** for ohchinolal.

The second compound (**2**), $C_{36}H_{42}O_8$, mp 184—185 °C, $[\alpha]_D^{23}$ +64° (*c* 0.17, EtOH), was isolated in a 0.020% yield as colorless needles from the more polar fraction; it displayed spectral data similar to those of ohchinin acetate (**3**).[5] The only significant difference in their IR and ^{1}H NMR data was the replacement of the acetoxyl group in **3** by the secondary hydroxyl group [ν_{max} 3450 cm^{-1}; δ=2.46 (d, J=8.9 Hz, OH) and 3.75 (ddd, J=8.9, 3.0, and 3.0 Hz, H_3)] in **2**. This relationship was verified by the acetylation of **2**, which gave the acetate (**3**). Thus, the name "Ohchinin," which we reserved for the natural precursor of **3** in the previous paper,[5] must be assigned to Compound **2**.

Experimental

All the mps were determined on a Mitamura micro-melting-point apparatus and are uncorrected. The IR and UV spectra were recorded on a JASCO model A-202 spectrophotometer and a Hitachi 340 spectrophotometer respectively. The ^{1}H and ^{13}C NMR spectra were taken on a Bruker WH-400 instrument in $CDCl_3$ solutions, with TMS as the internal standard. A JASCO apparatus, model DIP-140, was used for the measurement of the rotations. Column chromatography was performed using neutral alumina (Merck, activity III) and silicic acid (Wakogel C-200).

Isolation. The air-dried and milled fruit (2 kg) of *Melia azedarach* L. *var. japonica* Makino collected in Tokushima City in February, 1980, was extracted with methanol (6 l) at room temperature for two weeks. The methanol extract was then concentrated up to about 0.5 l, water (0.1 l) was added, and it was washed with petroleum ether The aqueous methanol layer was diluted with water (0.5 l) and then extracted with dichloromethane. The dichloromethane layer was washed with water, dried over $MgSO_4$, and evaporated to dryness. Then the residue (50 g) was subjected to chromatography over neutral alumina (1 kg), eluting with benzene–ethyl acetate mixtures, the ethyl acetate increasing from 10 to 70%. Elution with 30% ethyl acetate in benzene gave a fraction (20 g) which was rechromatographed over silicic acid (600 g), using 60% ethyl acetate in hexane as the eluent, to yield ohchinolal (**1**) (380 mg). Subsequent elution with 80% ethyl acetate in hexane afforded ohchinin (**2**) (485 mg).

Ohchinolal (1). The crude material was recrystallized from ether to give colorless prisms (250 mg); mp 163—164.5 °C, $[\alpha]_D^{23}$ +52° (*c* 0.22, EtOH); IR (KBr) 3475, 3150, 1730, 1710, 1690, 1645, 1500, 1250, 1040, 970, and 875 cm^{-1}; UV (EtOH) 215 nm (ε 15000); ^{1}H NMR (see Table 1); ^{13}C NMR (see Table 2); MS (70 eV) *m/z* (rel intensity) 612 (M^+, 22), 584 ($M^+ - CO$, 2), 552 ($M^+ - AcOH$, 6), 529 ($M^+ - C_5H_7O$, 12), 314 (13), 273 (39), 231 (69), 174 (38), 83 ($C_5H_7O^+$, 81), 55 (86), and 43 (100). Found: *m/z* 612.2955. Calcd for $C_{34}H_{44}O_{10}$: M, 612.2934.

Ohchinin (2). The crude substance was recrystallized from methanol to yield colorless needles (400 mg); mp 184—185 °C, $[\alpha]_D^{23}$ +64° (*c* 0.17, EtOH); IR (KBr) 3450, 3140, 1725, 1695, 1625, 1570, 1300, 1264, 1160, 865, 795, and 770 cm^{-1}; UV (EtOH) 215 and 276 nm (ε 22000 and 19000 respectively); ^{1}H NMR (see Table 1); ^{13}C NMR (see Table 2); MS (70 eV) *m/z* (rel intensity) 602 (M^+, 65), 587 ($M^+ - CH_3$, 3), 471 ($M^+ - C_9H_7O$, 12), 283 (73), 259 (20), 230 (16), and 131 ($C_9H_7O^+$, 100). Found: *m/z* 602.2906. Calcd for $C_{36}H_{42}O_8$: M, 602.2933.

The Oxidation of 1 with Pyridinium Chlorochromate. To a suspension of pyridinium chlorochromate (100 mg) in dichloromethane (2 ml) we added a solution of **1** (20 mg) in dichloromethane (0.4 ml). The mixture was stirred for 5 h at room temperature. After dilution with dichloromethane (10 ml), the solution was shaken with a 0.1 M (1 M=1 mol dm^{-3}) aqueous NaOH solution (10 ml) and then separated. The organic layer was washed with water, dried over $MgSO_4$, and evaporated to dryness. The product was chromatographed over silicic acid (3 g), with 50% ethyl acetate in hexane as the eluent, to yield an enone (**5**) as a viscous oil (8 mg); IR ($CHCl_3$) 1730, 1670, 1610, 1490, 1220, 1020, 900, and 865 cm^{-1}; UV (EtOH) 220 nm (ε 9800); ^{1}H NMR ($CDCl_3$) δ= 1.30, 1.31, and 1.42 (3H each, s, 29-, 19-, and 30-H_3), 1.72 (3H, d, J=1.4 Hz, 18-H_3), 1.99 (3H, s, Ac), 2.07 (1H, ddd, J= 12.2, 8.1, and 8.1 Hz, 16β-H), 2.23 (1H, dd, J=12.2 and 6.5 Hz, 16α-H), 2.49 (1H, dd, J=15.7 and 4.9 Hz, 11-H_a), 2.56 (1H, dd, J=4.9 and 4.9 Hz, 9-H), 2.63 (1H, dd, J=15.7 and 4.9 Hz, 11-H_b), 3.57 (3H, s, OCH_3), 3.58 (1H, d, J=12.2 Hz, 5-H), 3.67 (1H, br d, J=8.1 Hz, 17-H), 4.12 (1H, d, J=2.8 Hz, 7-H), 5.27 (1H, dd, J=12.2 and 2.8 Hz, 6-H), 5.49 (1H, m, 15-H), 5.99 (1H, d, J=10.3 Hz, 2-H), 6.99 (1H, d, J=10.3 Hz, 1-H), 6.22, 7.21, and 7.34 (1H each, br s, 22-, 21-, and 23-H), and 9.35 (1H, s, 28-H); MS (70 eV) *m/z* (rel intensity) 510 (M^+, 9), 482 ($M^+ - CO$,9), 422 ($M^+ - CO - AcOH$, 3), 259 (14), 247 (21), 231 (36), and 43 (100). Found: *m/z* 510.2326. Calcd for $C_{29}H_{34}O_8$: M, 510.2254. The combined aqueous solution was acidified with 3 M hydrochloric acid and extracted with ether. The ethereal solution was washed with saturated brine, dried over $MgSO_4$, and evaporated to dryness to give an acidic fraction (4 mg), which was found to contain tiglic acid by means of TLC and MS comparison with an authentic sample [MS (70 eV) *m/z* (rel intensity) 100 (M^+, 96) 85 ($M^+ - CH_3$, 34), 82 ($M^+ - H_2O$, 32), and 55 ($M^+ - COOH$, 100)].

Acetylation of **2**. A solution of **2** (8 mg) in acetic anhydride (0.1 ml) and pyridine (0.4 ml) was allowed to stand at room temperature overnight and then worked up in the usual way. The product was recrystallized from ether–dichloromethane to give an acetate as colorless prisms (7 mg); it was identified by mixed mp, IR, and ^{1}H NMR comparison with ohchinin acetate (**3**) [mp 223—226 °C; IR (KBr) 3160, 3060, 1735, 1695, 1640, 1585, 1500, and 875 cm^{-1}; ^{1}H NMR: see Ref. 5].

References

1) Part VI in the series of "Limonoids from *Melia azedarach* L. *var. japonica* Makino." Part V: see M. Ochi, H. Kotsuki, M. Ido, H. Nakai, M. Shiro, and T. Tokoroyama, *Chem. Lett.*, **1979**, 1137.

2) J. Polonsky, Z. Varon, B. Arnoux, C. Pascard, G. R. Pettit, J. M. Schmidt, and L. M. Lange, *J. Am. Chem. Soc.*, **100**, 2575 (1978); J. Polonsky, Z. Varon, B. Arnoux, C. Pascard, G. R. Pettit, and J. M. Schmidt, *ibid.*, **100**, 7731 (1978); J. Polonsky, Z. Varon, Ch. Marazona, B. Arnoux, G. R. Pettit, J. M. Schmidt, M. Ochi, and H. Kotsuki, *Experientia*, **35**, 987 (1979); S. D. Jolad, J. J. Hoffmann, K. H. Schram, J. R. Cole, M. S. Tempesta, and R. B. Bates, *J. Org. Chem.*, **46**, 641 (1981).

3) W. Kraus and R. Cramer, *Justus Liebigs Ann. Chem.*, **1981**, 181; M. Nakatani, J. C. James, and K. Nakanishi, *J. Am. Chem. Soc.*, **103**, 1228 (1981).

4) Professor I. Kubo, University of California, Berkeley, personal communication.

5) M. Ochi, H. Kotsuki, T. Kataoka, T. Tada, and T. Tokoroyama, *Chem. Lett.*, **1978**, 331.

6) W. Kraus and M. Bokel, *Chem. Ber.*, **114**, 267 (1981), and the references cited therein.

7) R. Henderson, R. McCrindle, A. Melera, and K. H. Overton, *Tetrahedron*, **24**, 1525 (1968).

8) V. P. Gullo, I. Miura, K. Nakanishi, A. F. Cameron, J.D. Connolly, F.D. Duncanson, A.E. Harding, R. McCrindle, and D. A. H. Taylor, *J. Chem. Soc., Chem. Comm.*, **1975**, 345, and the references cited therein.