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JACQUINONIC ACID, AN ANT-REPELLENT TRITERPENOID FROM *JACQUINIA PUNGENS*

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Key Word Index—*Jacquinia pungens*; Theophrastaceae; triterpenoid; oleanane; ant-repellent.

Abstract—The isolation and structure elucidation of jacquinonic acid, an ant-repellent triterpenoid from *Jacquinia pungens*, are described. This compound is a highly functionalized derivative of the oleanane skeleton, which contains both the uncommon C-13, 28 ether linkage and an α,β-unsaturated 3-keto group.

INTRODUCTION

Recent ecological studies have shown that some native plant species escape attack by the broadly polyphagous leafcutter ants (Hymenoptera, Formicidae, Attini), even though these insects in aggregate are the most destructive herbivores in the tropical Americas [1, 2]. Chemical investigations of plants that escape leafcutter attack have led to the isolation of ant-repellent sesquiterpenoids [3, 4] and triterpenoids [5]. In this paper we report the isolation of a new ant-repellent triterpenoid from *Jacquinia pungens*. This is a species which would appear especially vulnerable to leafcutter attack, for it retains its leaves throughout the dry season when most other deciduous species have shed their leaves and the number of available plant species is at a minimum. Nevertheless, *J. pungens* is rarely attacked by these ants.

RESULTS AND DISCUSSION

J. pungens leaves were collected from Costa Rica in January 1983, at the height of the dry season. The dried leaves were extracted successively with chloroform and then with ethanol. Both extracts were bioassayed using a procedure developed in our laboratory [1] for measuring the ant-repellency of various chemicals. In this case, the ant-repellent activity was found to reside in the chloroform extract. This extract was further partitioned into non-polar (hexane) and polar (50% aqueous methanol) fractions. After concentration the polar fraction consisted of a gummy material which showed significant activity. Final purification of this material by column chromatography on silica gel yielded the active compound **1**.

The molecular formula for **1** was established as $C_{30}H_{44}O_5$ by the high-resolution mass spectrum. Its IR spectrum showed absorption bands characteristic of an α,β-unsaturated carbonyl and a carboxyl group. The significant features of the ^1H NMR spectrum included six aliphatic methyl groups and two isolated AB spin systems, one due to the unsaturated ketone and the second indicative of geminal coupling in a $-CH_2O-$ group. The remaining oxygen was contained in a secondary hydroxyl group, as indicated by the resonance of its geminal proton. The ^{13}C NMR spectrum (Table 1) was compatible with these data. It confirmed the presence of an α,β-unsaturated carbonyl group, a carboxyl group and six methyl groups, and clearly showed *three* signals for sp^3 carbons bearing oxygen substituents. These spectral data taken together indicated that **1** is a pentacyclic triterpene with a cyclic ether moiety.

After comparison of these spectral data with those from a variety of known triterpenoids, we assigned this compound an oleanane skeleton with the functionality shown in structure **1**. Comparison of the carbon resonances of **1** with those assigned to the A- and B-rings of 3-oxoolean-1,18-diene (**4**) [6] and glochidone [7] was useful in establishing the position of the α,β-unsaturated carbonyl group as well as assigning carbon resonances in the A- and B-rings. Within the carbon spectrum reported for saikogenin G (**5**) [8], which possesses a 13,28-ether linkage and a C-16 hydroxyl group, the resonances assigned to the D-ring agree very well with a subset of the resonances found in the spectrum of **1**. This agreement supports inclusion of a cyclic ether linkage between C-13 and C-28, and placement of the secondary hydroxyl group at the C-16 position. To obtain further evidence for these conclusions, the methyl ester (**2**) derived from **1** was oxidized to the analogous ketone. The ^{13}C resonances of C-14, C-15, C-17 and C-28 shifted as expected upon oxidation of the alcohol to the ketone.

Once assigned to C-16, the stereochemistry of the secondary alcohol can be inferred from the NMR data. A width at half-height of 7 Hz for the hydrogen geminal to the hydroxyl group requires an equatorial hydrogen and an axial hydroxyl group [9]. Furthermore, the resonance of the C-14 methyl group is sensitive to the stereochemistry of the hydroxyl group at C-16 [8], and the value observed in this case is consistent with an axial hydroxyl group.

Location of the carboxyl group at C-20 is clear from the ^{13}C NMR data, and these data can also be used to determine the stereochemistry of this substituent [5]. By comparison with the spectra of β-amyrin [5], saikogenin G [8] and its dehydro derivative, a C-30 placement of this substituent can be determined since the ^{13}C resonances

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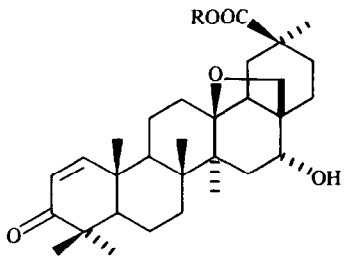
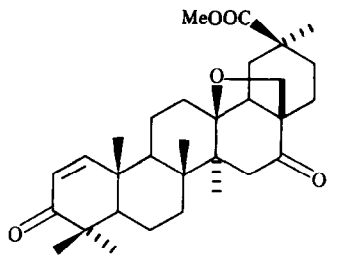


1 R = H
2 R = Me



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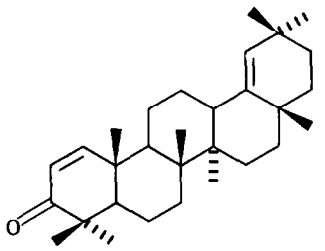

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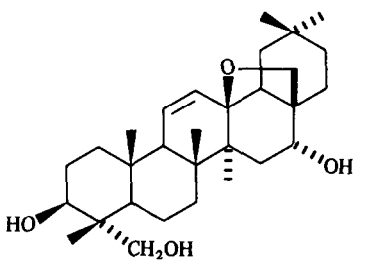

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assigned to the carbons in the E-ring of **2** compare favourably with those reported for the C-30 ester derivative of β-amyrin. Therefore, the complete structure of jacquinonic acid is 13β,28-epoxy-16α-hydroxy-3-oxo-olean-2-en-30-oic acid (**1**).

While there are many known β-amyrin derivatives, including one previously identified in *Jacquinia* [10], jacquinonic acid contains the uncommon 13β,28-epoxy functional group, as well as an unusually high density of functional groups overall. Which of these are essential for the observed biological activity is not yet clear, but this compound apparently has some value as a defensive agent with respect to discouraging leafcutter ant attack. The bioassay consists of a forced-choice test between treated and control food flakes. A concentration of 5.0 mg 1/ml, corresponding to an approximate final concentration of 100 μg/flake, results in a significant preference for the untreated materials ($P < 0.05$).

EXPERIMENTAL

Mps are uncorr. The IR spectra were obtained as Nujol mulls. The ^{1}H NMR spectra were obtained using $CDCl_3$ as solvent. Chemical shifts are reported in ppm downfield from Me_4Si. The broad-band decoupled and delayed-decoupling ^{13}C NMR spectra were obtained on a Bruker HX-90E instrument. Chemical shifts are reported in ppm downfield from (Me_4Si with $CDCl_3$ as both the solvent and the internal standard (77.0). Low-resolution MS were recorded with a Hewlett–Packard 5985B instrument operating at 70 eV in the electron impact mode. Only selected ions are reported here. High-resolution MS were obtained on an AIE MS-902 instrument at Cornell University, Mass Spectrometry Laboratory.

Isolation. The isolation sequence was guided by a bioassay [1] that measures repellency by monitoring ant choices among an array of treated and control food flakes. The significance of the T/C ratio is assessed by a binomial test.

Approximately 1.1 kg of *J. pungens* leaves (collected at Santa Rosa, Costa Rica in January 1983) were extracted successively with 2 l. $CHCl_3$ and then with 2 l. 95% EtOH in a Soxhlet for 24 hr. Both extracts were concentrated in vacuo and bioassayed. Ant-repellent activity was associated with the $CHCl_3$ extract ($P < 0.001$). This extract (38.6 g) was divided into polar and non-polar fractions by partitioning with hexane–50% aq. MeOH (2:1), and upon evapn of the solvents, 35.0 g (from hexane) and 3.1 g (from aq. MeOH) of gummy materials were obtained. Each fraction was bioassayed but only the polar fraction showed significant activity ($P < 0.001$).

CC of the polar fraction on silica gel (31 g, $CHCl_3$–MeOH gradient) yielded an active compound (45 mg) pure by TLC analysis.

Jacquinonic acid. Mp 315–316°; IR ν cm^{-1}: 1690, 1675; ^{1}H NMR: δ7.16 (1H, *d*, J = 10.1 Hz), 5.84 (1H, *d*, J = 10.1 Hz), 4.07 (1H, *br s*, $W_{1/2}$ = 7 Hz), 3.52 (1H, *d*, J = 7.3 Hz), 3.23 (1H, *d*, J = 7.3 Hz), 2.5–1.27 (19H, *m*), 1.25 (3H, *s*), 1.23 (6H, *s*), 1.15 (3H, *s*), 1.10 (3H, *s*), 1.09 (3H, *s*); ^{13}C NMR: see Table 1; EIMS *m/z* (rel. int.): 484 $[M]^+$ (9), 466 (4), 454 (2), 422 (5), 393 (7), 279 (16), 266 (23), 219 (23), 205 (30), 203 (32), 189 (32), 185 (28), 135 (58), 121 (54), 107 (100); high-resolution MS: found: *m/z* 484.3179; calc. for $C_{30}H_{44}O_5$: *m/z* 484.3188.

Methyl ester of jaquinonic acid. Excess CH_2N_2 was added to an Et_2O soln of jacquinonic acid (20 mg, 4.1×10^{-2} mmol) and the resulting soln was left at room temp. overnight. Evapn of the volatile materials gave the methyl ester **2**: 20.1 mg (98%); mp 230–232°; ^{1}H NMR: δ7.14 (1H, *d*, J = 10.1 Hz), 5.84 (1H, *d*, J = 10.1 Hz), 4.08 (1H, *br s*, $W_{1/2}$ = 7 Hz), 3.68 (3H, *s*), 3.53 (1H, *d*, J = 7.3 Hz), 3.24 (1H, *d*, J = 7.3 Hz), 2.48–1.26 (19H, *m*), 1.25 (3H, *s*), 1.23 (6H, *s*), 1.15 (3H, *s*), 1.10 (3H, *s*), and 1.09 (3H, *s*);

Table 1. ^{13}C Chemical shifts of **1**, its derivatives and some related oleananes

C	1	4	5	2	3
1	160.0 *d*	159.8 *d*	38.0 *t*	159.8 *d*	159.0 *d*
2	125.0 *d*	125.2 *d*	26.1 *t*	125.3 *d*	125.0 *d*
3	205.6 *s*	205.0 *s*	75.6 *d*	205.6 *s*	205.1 *s*
4	44.7 *s*	44.6 *s*	42.0 *s*	44.7 *s*	44.7 *s*
5	53.3 *d*	53.8 *d*	49.2 *d*	53.3 *d*	53.3 *d*
6	18.4 *t*	19.9 *t*	17.7 *t*	18.4 *t*	18.3 *t*
7	35.2 *t*	34.1 *t*	31.1 *t*	35.2 *t*	33.1 *t*
8	39.3 *s*	39.5 *s*	41.4 *s*	39.3 *s*	39.2 *s*
9	44.1 *d*	45.2 *d*	52.5 *d*	44.1 *d*	44.2 *d*
10	42.9 *s*	41.6 *s*	36.3 *s*	42.9 *s*	43.5 *s*
11	19.1 *t*	21.4 *t*	132.6 *d*	19.1 *t*	18.9 *t*
12	36.8 *t*	26.1 *t*	130.5 *d*	36.8 *t*	36.5 *t*
13	86.6 *s*	38.6 *d*	85.1 *s*	86.6 *s*	86.0 *s*
14	43.5 *s*	43.5 *s*	43.0 *s*	43.5 *s*	45.0 *s*
15	36.7 *t*	27.5 *t*	34.7 *t*	36.7 *t*	50.1 *t*
16	77.3 *d*	37.6 *t*	77.3 *d*	77.5 *d*	213.0 *s*
17	45.1 *s*	34.3 *s*	44.9 *s*	45.4 *s*	56.1 *d*
18	52.6 *d*	142.3 *s*	50.6 *d*	52.6 *d*	55.3 *s*
19	32.7 *t*	129.9 *d*	38.0 *t*	32.1 *t*	31.4 *t*
20	44.2 *s*	32.3 *s*	31.7 *s*	44.2 *s*	45.3 *s*
21	33.5 *t*	33.3 *t*	36.5 *t*	33.5 *t*	31.8 *t*
22	31.9 *t*	37.4 *t*	30.5 *t*	31.9 *t*	26.0 *t*
23	27.8 *q*	27.8 *q*	70.3 *t*	27.8 *q*	27.7 *q*
24	21.4 *q*	21.4 *q*	11.1 *q*	21.4 *q*	21.4 *q*
25	18.9 *q*	19.0 *q*	18.3 *q*	18.9 *q*	18.8 *q*
26	19.2 *q*	16.7 *q*	19.0 *q*	19.2 *q*	18.9 *q*
27	18.4 *q*	14.7 *q*	17.9 *q*	18.4 *q*	21.4 *q*
28	77.5 *t*	25.3 *q*	77.3 *t*	77.6 *t*	75.1 *t*
29	28.3 *q*	31.3 *q*	33.4 *q*	28.3 *q*	28.3 *q*
30	183.0 *s*	29.2 *q*	24.2 *q*	177.9 *s*	176.8 *s*
$-CO_2\underline{C}H_3$				51.7 *q*	51.7 *q*

EIMS *m/z* (rel. int.): 498 $[M]^+$ (2), 468 (2), 450 (3), 407 (1), 392 (1), 293 (5), 280 (8), 233 (16), 221 (22), 205 (22), 203 (25), 189 (28), 185 (24), 137 (55), 135 (61), 119 (49), 107 (100).

Oxidation of **2**. To a stirred soln of the methyl ester **2** (18 mg, 3.6×10^{-2} mmol) in dry DMF (1 ml) was added pyridinium dichromate (98.4 mg, 0.26 mmol), and the resulting soln was stirred overnight at room temp. The reaction mixture was then poured into ice H_2O (10 ml), and this mixture was stirred for 30 min, extracted with Et_2O (3 × 40 ml), and the combined Et_2O extracts were dried. Evapn of the Et_2O gave the 16-oxotriterpenoid **3**: 15.5 mg (87%); mp 213–215°; ^1H NMR: δ 7.13 (1H, *d*, *J* = 10.2 Hz), 5.83 (1H, *d*, *J* = 10.2 Hz), 3.88 (1H, *d*, *J* = 8.3 Hz), 3.68 (3H, *s*), 3.38 (1H, *d*, *J* = 8.3 Hz), 2.73 (1H, *d*, *J* = 16 Hz), 2.24 (1H, *d*, *J* = 16 Hz), 2.12–1.26 (19H, *m*), and 1.32, 1.18, 1.14, 1.12, 1.10, 1.04 (each 3H, *s*); EIMS *m/z* (rel. int.): 466 $[M-30]^+$ (2), 292 (9), 279 (6), 247 (11), 235 (1), 205 (5), 203 (7), 189 (8), 187 (5), 137 (10), 43 (100).

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