Separation and Determination of Chemical Constituents in the Roots of *Rhus javanica* L. var. *roxburghiana*

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From the roots of *Rhus javanica* L. var. *roxburghiana*, totally thirty-seven known compounds have been isolated and identified. Their structures were elucidated based on their spectral analysis as well as comparison with authentic samples. These compounds were grouped to be fifteen triterpenoids, five steroids, two lignans, two flavonoids, nine phenolics, and four other aromatic derivatives. Their cytotoxicities toward two cell lines NUGC-3 and HONE-1 were also evaluated.

Keywords: *Rhus javanica* L. var. *roxburghiana*; Anacardiaceae; Roots; Cytotoxicity; NUGC-3; HONE-1.

INTRODUCTION

There are five species of *Rhus* (Anacardiaceae) found in Taiwan. *R. javanica* L. var. *roxburghiana*, a small-sized deciduous tree, is widely distributed in thickets and secondary forests at low altitudes throughout this island.[1] Its roots have been used in folk medicines as antitussives, and for the treatments of anasarca, jaundice, and snake bite.[2] The phytochemical studies on this plant have been reported earlier, and which have resulted in the isolation and characterization of flavonoids,[3-4] triterpenoids,[5-7] phenolics,[3,8] one tannin[9] and one aromatic alkane.[5] Recently, it has been shown from our preliminary pharmacological experiments that the ethyl acetate layer of the root extracts of this plant exhibited significant anti-proliferation activities on four cell lines including 59T (hepatoma), DLD-1 (colon cancer), HONE-1 (nasopharyngeal carcinoma), and SCM1 (stomach carcinoma). The roots may contain bioactive agents with anti-proliferation activities worth investigating phytochemically. Therefore, a series of phytochemical examinations on the ethyl acetate layer of the root extracts of this plant was thus undertaken and has led to the isolation and characterization of thirty seven known compounds **1**-**37**. This paper deals with the isolation and structural elucidation of these compounds as well as their cytotoxicities.

RESULTS AND DISCUSSION

The methanolic extracts of the roots of *R. javanica* L. var. *roxburghiana* were concentrated to give a residue which was subjected to partition with *n*-hexane and water. The aqueous layer was further partitioned with EtOAc and *n*-BuOH, successively. The combined EtOAc soluble fractions were then separated using Si-open column chromatography and HPLC repeatedly to yield thirty seven components: betulonic acid (**1**),[10] betulinic acid (**2**),[11] betulin (**3**),[12] lantabetulic acid (**4**),[13] 3-oxoolean-18-en-28-oic acid (**5**),[14] 3β-hydroxyolean-18-en-28-oic acid (**6**),[15] 3-oxo-6β-hydroxyolean-18-en-28-oic acid (**7**),[16] semimoronic acid (**8**),[13] 3-*O*-methyl semimoronic acid (**9**),[17] 3-oxoolean-12-en-28-oic acid (**10**),[18] oleanolic acid (**11**),[19] lantanolic acid (**12**),[20] 3-oxotirucalla-7,24-dien-21-oic acid (**13**),[21] dipterocarpol (**14**),[22] 3β-hydroxy-22,23,24,25,26,27-hexanordammaran-20-one (**15**),[23] β-sitosterol (**16**),[24] stigmast-4-en-3-one (**17**),[25] stigmast-4-ene-3,6-dione (**18**),[26] stigmastane-3,6-dione (**19**),[25] stigmast-7-en-3-ol (**20**),[27] pinoresinol (**21**),[28] 4-oxopinoresinol (**22**),[29] 4′,5,7-trihydroxyflavanone (**23**),[30] *trans*-3,4′,7-trihydroxyflavanone (**24**),[31] vanillin (**25**),[32] methyl ferulate (**26**),[33] 3,5-dihydroxytoluene (**27**),[34] 4-hydroxy-3,5-dimethoxybenzaldehyde (**28**),[35] methyl gallate (**29**),[36] 2,6-dimethoxy[1,4]benzoquinone (**30**),[37] 3,4,5-trimethoxyben-

Dedicated to Professor Ching-Erh Lin on the Occasion of his 66th Birthday and his Retirement from National Taiwan University

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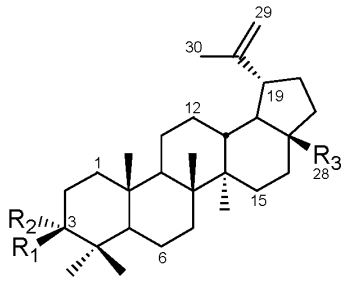
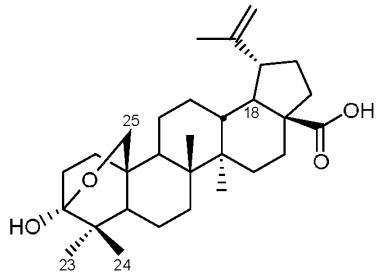


	R_1	R_2	R_3
1	=O		$-CO_2H$
2	-OH	-H	$-CO_2H$
3	-OH	-H	$-CH_2OH$



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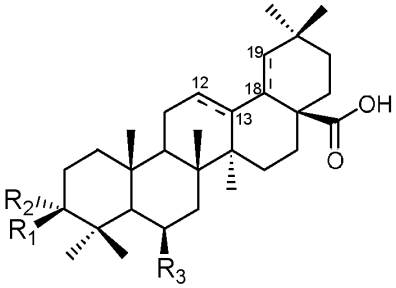
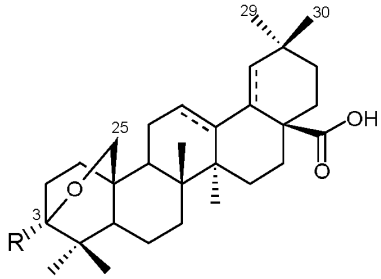
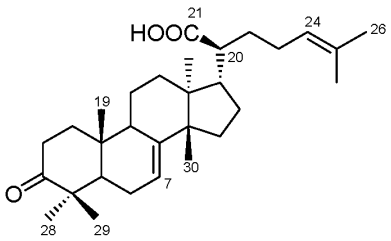


	R_1	R_2	R_3
5 (Δ^{18})	=O		-H
6 (Δ^{18})	-OH	-H	-H
7 (Δ^{18})	=O		-OH
10 (Δ^{12})	=O		-H
11 (Δ^{12})	-OH	-H	-H



	R
8 (Δ^{18})	-OH
9 (Δ^{18})	-OMe
12 (Δ^{12})	-OH



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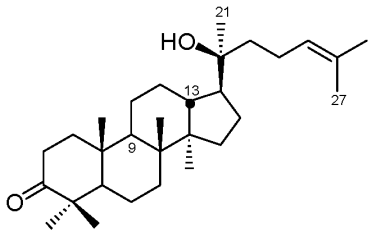

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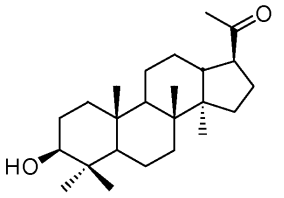
zyl alcohol (**31**),[38] gallic acid (**32**),[39] ficusol (**33**),[40] 2-hydroxy-6-pentadec-8(*Z*)-enylbenzoic acid (**34**),[41] 5-formylmellein (**35**),[42] 5-hydroxymethylmellein (**36**),[42] and alkyls caffeate (**37**).[43]

When comparing the spectral data of **4** with those of **2** it was suggested **4** had the typical lupane skeleton except that the ^{1}H-NMR of **4** showed two methylene proton signals (H_2-25) at δ 3.71 (dd, J = 8.8, 1.6 Hz) and δ 4.21 (dd, J = 8.8, 2.8



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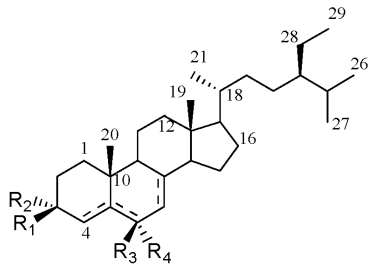
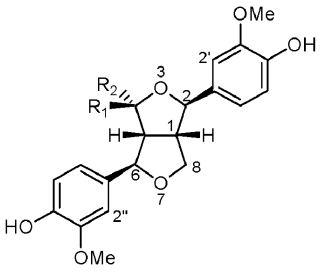
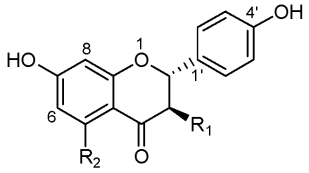


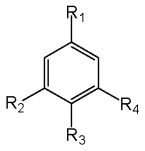
	R_1	R_2	R_3	R_4
16 (Δ^5)	-OH	-H	-H	-H
17 (Δ^4)	=O		-H	-H
18 (Δ^4)	=O		=O	
19	=O		=O	
20 (Δ^7)	-OH	-H	-H	-H



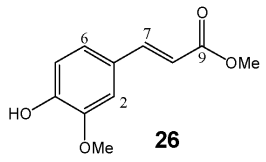
	R_1	R_2
21	-H	-H
22	=O	



	R_1	R_2
23	-H	-OH
24	-OH	-H



	R_1	R_2	R_3	R_4
25	-CHO	-H	-OH	-OMe
27	-Me	-OH	-H	-OH
28	-CHO	-OMe	-OH	-OMe
29	$-CO_2Me$	-OH	-OH	-OH
31	$-CH_2OH$	-OMe	-OMe	-OMe
32	$-CO_2H$	-OH	-OH	-OH



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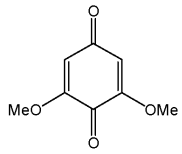

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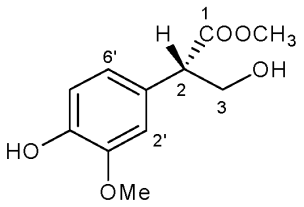
Hz) having W-shaped ^{1}H-^{1}H correlations with those of H_α-1 and H-5, respectively. The orientation of H_2-25 was assigned to be β-form by a NOESY experiment. Thus, the structure of **4** was determined to be lantabetulic acid, which was isolated originally from *Lantana camara*[13] and also found previously in *R. javanica*.[5] Compounds **8**, **9** and **12** possessed spectroscopic data closely comparable to a oleanolic acid skeleton except that a H_3-25 singlet was substituted by a oxygenated methylene functionality which bridged between C-3 and C-10, the same as in **4**. The ^{1}H NMR spectra also showed characteristic signals for the olefinic protons in **8** (δ 5.13, s, H-19), **9** (δ 5.12, s, H-19), and **12** (δ 5.29, t, J = 2.8 Hz, H-12), and the location of the double bonds was deduced to be at C-18 and C-12, respectively, which was confirmed by interpreting their HMBC spectra. Further analysis of all the 2D NMR data of **8**, **9** and **12** allowed the complete assignments of their ^{1}H and ^{13}C NMR data. Accordingly, **8**, **9**, and **12** were assigned to be semimoronic acid, 3-*O*-methyl semimoronic acid, and lantanolic acid, respectively. Compounds **25**, **27**, **28**, **29**, **31**, and **32** were identified as toluene derivatives with higher oxygenated on phenyl protons at C-3, -4, -5 and benzylic positions. The presence of 1,3,4,5-tetra-substituted aro-



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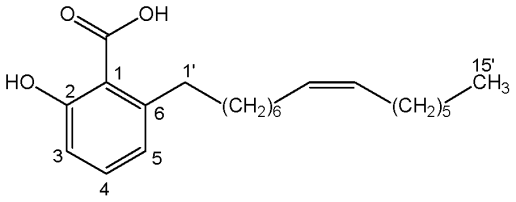

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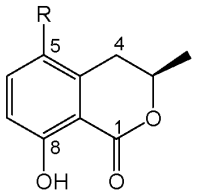
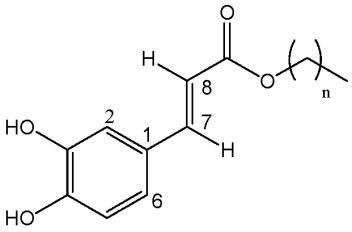


35 -CHO
36 -CH_2OH



n=21(4%), 23(16%), 25(68%), 27(12%)

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matic moiety in **28**, **29**, **31**, and **32** was supported by their ^{1}H NMR spectra, in which two phenyl protons are symmetrical. Compounds **25** and **27** possessing 1,2,5- and 1,3,5-phenyl protons, respectively, were interpreted by their coupling patterns. Among them, gallic acid (**32**) and its derivatives were the compounds most widely distributed in higher plants to be the component of hydrolysable tannins, flavonoids, or many other chemicals, and often exhibit important therapeutic activities. ^{1}H NMR spectrum of **34** showed signals for a 1,2,3-trisubstituted aromatic moiety, and an ABC system at δ 6.74 (d, J = 7.2 Hz, H-5), δ 6.84 (d, J = 8.0 Hz, H-3), and δ 7.31 (dd, J = 8.0, 7.2 Hz, H-4) were observed. Besides, this spectrum also displayed signals for an unsaturated long-chain alkyl functionality, one terminal methyl triplet at δ 0.88 (H_3-15′), four methylene at δ 1.59 (m, H_2-2′), 2.00 (m, H_2-7′, -10′), 2.96 (t, J = 7.6 Hz, H_2-1′), and an olefinic multiplet at δ 5.33 (H-8′, -9′). The location of the double bond was further confirmed to be at C-8′ by an oxidative cleavage experiment, and its configuration should be *Z*-form as evidenced by two signals at δc 26.9 (C-7′) and 27.2 (C-10′) below δc 30 in the ^{13}C NMR spectrum. After a complete assignment of its ^{1}H and ^{13}C NMR data, **34** was concluded to be 2-hydroxy-6-pentadec-8(*Z*)-enyl-benzoic acid, quite similar to the structure of 6-pentadecylsalicylic acid isolated from *R. javanica* previously.[5] 6-Pentadecylsalicylic acid found to be an antithrombin agent and prolonged the clotting time in a dose-dependent manner, however, was not found in this study. Whether **34** can exert the same bioactivity or not remained to be studied. Compound **37** were isolated as a mixture of four alkyl caffeates using HPLC with a solution of 30:70 ethyl acetate/hexane as eluent. The lengths of their side chains were confirmed to be C_{21}, C_{23}, C_{25}, and C_{27} by mass analysis.

All the identified compounds were grouped to be fourteen different skeletons: lupane (**1**, **2**, **3**, and **4**), oleanane (**5**, **6**, **7**, **8**, **9**, **10**, **11**, and **12**), tirucallane (**13**), dammaranoid (**14** and **15**), steroid (**16**, **17**, **18**, **19**, and **20**), lignan (**21** and **22**), flavonoid (**23** and **24**), and four aromatic alkanoid types including C_6 (**30**), C_6-C_1 (**25**, **27**, **28**, **29**, **31**, and **32**), phenylisopropanoid (**33**), phenylpropanoid (**26** and **37**), and other skeletons (**34**, **35**, and **36**).

Compounds with higher quantities were tested for their cytotoxicity using two cancer cell lines, NUGC-3 and HONE-1. At the concentration of 20 μg/mL, **29** was found to be the most cytotoxic compound, and the survival rates of NUGC-3 and HONE-1 were 13% and 24%, respectively. At the same concentration, compound **4** caused the NUGC-3 and HONE-1 cells to show 10% and 38% survival rates, respectively.

EXPERIMENTAL SECTION

The roots of *R. javanica* L. var. *roxburghiana* were collected at Kaohsiung, Taiwan in June, 2000. Melting points were collected using a Yanaco MP-53 apparatus. Optical Rotations were measured with a JASCO DIP-180 digital

polarimeter at room temperature. IR spectra were recorded on a Perkin-Elmer 983G spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AM-300 and DMX-500 instruments using tetramethylsilane (TMS) as internal standard. Chemical shifts are given in δ values (ppm) and coupling constants (*J*) are given in hertz (Hz). Electron-impact mass spectrum (EI-MS) and fast atomic bombard mass spectrum (FAB-MS) were obtained on JMS-HX 110 and JEOL JMS-HX 110 mass spectrometers, respectively. For thin-layer chromatography analysis, Kieselgel 60 F_{254} plates (Merck, Germany) were used. HPLC was performed on GBC LC-1440 and LDC Analytical-III liquid chromatographs with Lichrosorb Si-60 column (7 μm, 250 × 10 mm, Merck, Germany).

Extraction and Isolation

The air dried roots of *R. javanica* L. var. *roxburghiana* (18.0 kg) were extracted with 120 L MeOH three times (seven days each time) at room temperature. The combined extracts were evaporated under vacuum to give a residue, which was suspended in 8 L water and then partitioned with EtOAc and *n*-BuOH, successively. The EtOAc layer was evaporated to yield residues of 500 g, which were subjected to cytotoxicity tests first. At the concentration of 50 μg/mL, the cell viabilities of 59 T, DLD-1, HONE-1, and SCM1 were 19%, 56%, 16%, and 42%, respectively. Based on these findings, the ethyl acetate layer was then chromatographed by Si-column and HPLC, repeatedly. The eluent systems are combinations of *n*-hexane and EtOAc, EtOAc, EtOAc and MeOH in a step elution mode. After a series of separations, the components obtained were **1** (50 mg), **2** (1 mg), **3** (3 mg), **4** (6 mg), **5** (13 mg), **6** (2 mg), **7** (12 mg), **8** (12 mg), **9** (4 mg), **10** (2 mg), **11** (2 mg), **12** (9 mg), **13** (3 mg), **14** (14 mg), **15** (7 mg), **16** (70 mg), **17** (18 mg), **18** (9 mg), **19** (16 mg), **20** (6 mg), **21** (8 mg), **22** (3 mg), **23** (6 mg), **24** (7 mg), **25** (4 mg), **26** (14 mg), **27** (30 mg), **28** (4 mg), **29** (2.5 g), **30** (1 mg), **31** (1 mg), **32** (16 mg), **33** (2 mg), **34** (120 mg), **35** (3 mg), **36** (1 mg), and **37** (5 mg).

The physical and chemical data including major ^{1}H-NMR interpretations of compounds **1-37** were list as follows:

Betulonic acid (1)

IR ν_{max}: 3300-2500, 1706, 1697, 1645 cm^{-1}; mp: 243-244 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.89, 0.93, 0.95, 0.98, 1.03 (each 3H, s), 1.65 (3H, s, H-30), 2.3-2.5 (2H, m, H-2), 2.96 (1H, td, *J* = 10.8, 4.4 Hz, H-19), 4.57, 4.70 (each 1H, brs, H-29); EI-MS *m/z* (%): 454 (M^+, 58), 248 (64), 219 (42), 205 (76), 189 (88), 136 (100), 121 (90).

Betulinic acid (2)

IR ν_{max}: 3462, 3300-2500, 1690, 1646 cm^{-1}; mp: 300-301 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.73, 0.80, 0.91, 0.94, 0.95 (each 3H, s), 1.67 (3H, s, H-30), 2.97 (1H, td, *J* = 10.4, 4.8 Hz, H-19), 3.16 (1H, dd, *J* = 10.8, 5.4 Hz, H-3), 4.58, 4.71 (each 1H, brs, H-29); FAB-MS *m/z* (%): 457 (M^++H, 7), 307 (25), 154 (100), 136 (60).

Betulin (3)

IR ν_{max}: 3355, 1645 cm^{-1}; mp: 252-254 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.74, 0.80, 0.94, 0.96, 1.00 (each 3H, s), 1.66 (3H, s, H-30), 2.35 (1H, td, *J* = 10.6, 5.8 Hz, H-19), 3.16 (1H, dd, *J* = 11.0, 5.3 Hz, H-3), 3.31, 3.78 (each 1H, d, *J* = 10.8 Hz), 4.56, 4.66 (each 1H, brs); EI-MS *m/z* (%): 442 (M^+, 40), 411 (60), 203 (95), 189 (100), 95 (85).

Lantabetulic acid (4)

IR ν_{max}: 3372, 3300-2500, 1692, 1644 cm^{-1}; mp: 252-254 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.83, 0.93, 0.94, 1.00 (each 3H, s), 1.66 (3H, s, H-30), 2.96 (1H, td, *J* = 10.8, 4.8 Hz, H-19), 3.71 (1H, dd, *J* = 8.8, 1.6 Hz, H-25), 4.21 (1H, dd, *J* = 8.8, 2.8 Hz, H-25), 4.59, 4.70 (each 1H, brs); EI-MS *m/z* (%): 442 (M^+, 58), 411 (60), 203 (95), 189 (100), 95 (85).

3-Oxoolean-18-en-28-oic acid (5)

IR ν_{max}: 3300-2500, 1700, 1654 cm^{-1}; mp: 222 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.76, 0.92, 0.95, 0.97, 0.98, 0.99, 1.05 (each 3H, s), 2.4-2.5 (2H, m, H-2), 5.14 (1H, s, H-19); EI-MS *m/z* (%): 454 (M^+, 10), 248 (68), 235 (77), 191 (100), 190 (73).

3β-Hydroxyolean-18-en-28-oic acid (6)

IR ν_{max}: 3460, 3300-2500, 1694, 1653 cm^{-1}; mp: 271-273 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.74, 0.75, 0.84, 0.94, 0.95, 0.96, 0.98 (each 3H, s), 3.18 (1H, dd, *J* = 11.0, 5.4 Hz, H-3), 5.16 (1H, s, H-19); EI-MS *m/z* (%): 456 (M^+, 20), 248 (82), 203 (72), 189 (100), 163 (58).

3-Oxo-6β-hydroxyolean-18-en-28-oic acid (7)

IR ν_{max}: 3405, 3300-2500, 1701 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.72, 0.94, 0.97, 1.12, 1.32, 1.38, 1.42 (each 3H, s), 2.78 (1H, td, *J* = 15.2, 6.4 Hz, H-2), 4.44 (1H, brs, H-6), 5.14 (1H, s, H-19); EI-MS *m/z* (%): 470 (M^+, 10), 426 (42), 235 (64), 189 (100), 163 (56).

Semimoronic acid (8)

IR ν_{max}: 3412, 3300-2500, 1698 cm^{-1}; mp: 260-261 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.72, 0.85, 0.94, 0.95, 0.97, 1.00 (each 3H, s), 3.71 (1H, dd, *J* = 8.4, 1.6 Hz, H-25), 4.26 (1H, dd, *J* =

8.4, 2.4 Hz, H-25), 5.13 (1H, s, H-19); EI-MS *m/z* (%): 470 (M^+, 10), 424 (24), 189 (84), 163 (100), 119 (80), 105 (95).

3-*O*-Methyl semimoronic acid (9)

IR ν_{max}: 3300-2500, 2929, 2861, 1700, 1606 cm^{-1}; mp: 235-238 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.72, 0.94, 0.94, 0.94, 0.96 (each 3H, s), 1.16 (1H, m, H_a-2), 2.16 (1H, m, H_b-2), 3.20 (3H, s, H-31), 3.72 (1H, dd, *J* = 8.4, 1.6 Hz, H-25), 4.26 (1H, dd, *J* = 8.4, 2.4 Hz, H-25), 5.12 (1H, s, H-19); EI-MS *m/z* (%): 484 (M^+, 20), 438 (22), 249 (28), 235 (23), 189 (82).

3-Oxoolean-12-en-28-oic acid (10)

IR ν_{max}: 3300-2500, 3082, 1697, 1387, 1367, 1271, 738 cm^{-1}; mp: 183-185 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.79, 0.88, 0.91, 1.01, 1.02, 1.06, 1.12 (each 3H, s), 2.34 (1H, ddd, *J* = 15.6, 6.8, 3.6 Hz, H_a-2), 2.52 (1H, ddd, *J* = 15.6, 11.6, 7.6 Hz, H_b-2), 2.81 (1H, dd, *J* = 9.6, 4.0 Hz, H-18), 5.28 (1H, t, *J* = 4.0 Hz, H-12); FAB-MS *m/z* (%): 455 (M^++H, 7), 409 (3), 391 (3), 248 (9), 154 (100).

Oleanolic acid (11)

IR ν_{max}: 3450, 3300-2600, 3005, 1696, 1388, 1037 cm^{-1}; mp: 273-275 °C; ^{1}H-NMR (CD_3OD) δ: 0.76 (1H, brd, *J* = 9.6 Hz, H-5), 0.78, 0.82, 0.91, 0.95, 0.95, 0.98, 1.16 (each 3H, s), 2.85 (1H, dd, *J* = 14.0, 4.0 Hz, H-18), 3.15 (1H, dd, *J* = 11.2, 4.8 Hz, H-3), 5.24 (1H, t, *J* = 3.6 Hz. H-12); FAB-MS *m/z* (%): 457 (M^++H, 1), 391 (3), 154 (100).

Lantanolic acid (12)

IR ν_{max}: 3462, 3300-2600, 2942, 1704, 1643 cm^{-1}; mp: 307-309 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.71, 0.88, 0.90, 0.95, 1.01, 1.10 (each 3H, s), 2.80 (1H, dd, *J* = 13.6, 4.4 Hz, H-18), 3.87 (1H, dd, *J* = 8.8, 1.6 Hz, H-25), 4.19 (1H, dd, *J* = 8.8, 2.8 Hz, H-25), 5.29 (1H, t, *J* = 2.8 Hz, H-12); EI-MS *m/z* (%): 470 (M^+, 35), 424 (38), 248 (38), 241 (100), 203 (96).

3-Oxotirucalla-7,24-dien-21-oic acid (13)

IR ν_{max}: 3382, 3300-2600, 1704, 1654 cm^{-1}; mp: 274-276 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.88, 0.98, 1.00, 1.04, 1.10 (each 3H, s), 1.57 (3H, s, H-26), 1.67 (3H, s, H-27), 2.73 (1H, td, *J* = 14.8, 4.8 Hz, H_β-2), 5.08 (1H, t, *J* = 6.8 Hz, H-24), 5.30 (1H, brs, H-7); EI-MS *m/z* (%): 454 (M^+, 35), 439 (100), 421 (64), 297 (82).

Dipterocarpol (14)

IR ν_{max}: 3482, 2954, 2875, 1705 cm^{-1}; mp: 133-134 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.87, 0.93, 0.99, 1.02, 1.07, 1.14, 1.62, 1.67 (each 3H, s), 5.11 (1H, t, *J* = 7.2 Hz, H-24); EI-MS *m/z* (%): 424 (M^+, 58), 355 (24), 205 (58), 109 (100).

3β-Hydroxy-22,23,24,25,26,27-hexanordammaran-20-one (15)

IR ν_{max}: 3398, 2938, 2869, 1708 cm^{-1}; mp: 133-134 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.75, 0.83, 0.85, 0.95, 0.96 (each 3H, s), 2.11 (3H, s, H-21), 2.56 (1H, td, *J* = 11.2, 6.4 Hz, H-17), 3.18 (1H, dd, *J* = 11.2, 5.2 Hz, H-3); EI-MS *m/z* (%): 360 (M^+, 20), 317 (48), 299 (100), 207 (48), 95 (63).

β-Sitosterol (16)

IR ν_{max}: 3428, 1640 cm^{-1}; mp: 144-146 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.66 (3H, s, H-18), 0.79 (3H, d, *J* = 6.5 Hz, H-27), 0.81 (3H, d, *J* = 6.5 Hz, H-26), 0.82 (3H, t, *J* = 7.2 Hz, H-29), 0.91 (3H, d, *J* = 6.4Hz, H-21), 1.00 (3H, s, H-19), 3.49 (1H, m, H-3), 5.33 (1H, d, *J* = 5.3 Hz, H-6); EI-MS *m/z* (%): 414 (M^+, 92), 396 (100), 81 (78).

Stigmast-4-en-3-one (17)

IR ν_{max}: 3040, 1683, 1380 cm^{-1}; mp: 84-86 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.68, 1.15 (each 3H, s), 0.81, 0.83 (each 3H, d, *J* = 6.3 Hz), 0.86 (3H, t, *J* = 7.2 Hz), 0.95 (3H, d, *J* = 6.2 Hz), 5.69 (1H, s); EI-MS *m/z* (%): 412 (M^+, 95), 397, 370, 289, 229, 124 (100).

Stigmast-4-ene-3,6-dione (18)

IR ν_{max}: 1671, 1622 cm^{-1}; mp: 172-173 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.70 (3H, s, H-18), 0.78 (3H, d, *J* = 6.8 Hz, H-27), 0.79 (3H, d, *J* = 7.0 Hz, H-26), 0.83 (3H, t, *J* = 7.0 Hz, H-29), 0.91 (3H, d, *J* = 6.5 Hz, H-21), 1.14 (3H, s, H-19), 6.15 (1H, s, H-4); EI-MS *m/z* (%): 426 (M^+, 100), 412 (54), 398 (58), 137 (82).

Stigmastane-3,6-dione (19)

IR ν_{max}: 1716, 1708, 1239 cm^{-1}; mp: 205-206 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.69 (3H, s), 0.82 (3H, d, *J* = 6.8 Hz), 0.84 (3H, d, *J* = 6.5 Hz), 0.85 (3H, t, *J* = 7.0 Hz), 0.93 (3H, d, *J* = 6.4 Hz), 0.96 (3H, s); EI-MS *m/z* (%): 428 (M^+, 88), 413 (10), 331 (9), 287 (22), 245 (59), 231 (19), 149 (100).

Stigmast-7-en-3-ol (20)

IR ν_{max}: 3422, 1450, 1377 cm^{-1}; mp: 151-152 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.51 (3H, s, H-18), 3.56 (1H, m, H-3), 5.14 (1H, brs, H-7); EI-MS *m/z* (%): 414 (M^+,100), 396 (36), 271 (48), 255 (92).

Pinoresinol (21)

IR ν_{max}: 3416, 2928, 2858, 1608 cm^{-1}; mp: 136-137 °C;

^{1}H-NMR ($CDCl_3$) δ: 3.10 (2H, m, H-1, H-5), 3.88 (6H, s, H_3-3′, H_3-3″), 3.94 (2H, dd, *J* = 9.1, 3.5 Hz, H_α-4, H_α-8), 4.23 (2H, dd, *J* = 9.1 Hz, 6.8 Hz, H_β-4, H_β-8), 4.72 (2H, d, *J* = 4.2 Hz, H-2, H-6), 6.80 (2H, dd, *J* = 8.4, 1.6 Hz, H-6′, H-6″), 6.87 (2H, d, *J* = 8.4 Hz, H-5′, H-5″), 6.87 (2H, d, *J* = 1.6 Hz, H-2′, H-2″); EI-MS *m/z* (%): 358 (M^+, 45), 205 (20), 151 (100), 137 (55), 131 (28).

4-Oxopinoresinol (22)

IR ν_{max}: 3431, 2944, 2855, 1762, 1605 cm^{-1}; mp: 127-128 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.24 (1H, m, H-1), 3.46 (1H, dd, *J* = 9.2, 4.0 Hz, H-5), 4.04 (1H, dd, *J* = 9.2, 4.4 Hz, H_α-8), 4.33 (1H, dd, *J* = 9.2, 6.8 Hz, H_β-8), 5.31 (1H, d, *J* = 4.0 Hz, H-6), 5.33 (1H, d, *J* = 4.0 Hz, H-2), 6.7-7.3 (6H, m, Ar-H); EI-MS *m/z* (%): 372 (M^+, 88), 163 (24), 151 (100), 131 (55).

4′,5,7-Trihydroxyflavanone (23)

IR ν_{max}: 3384, 1631, 1605, 1462 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.72 (1H, dd, *J* = 17.1, 3.0 Hz, H_a-3), 3.19 (1H, dd, *J* = 17.1, 12.9 Hz, H_b-3), 5.44 (1H, dd, *J* = 12.9, 3.0 Hz, H-2), 5.92 (1H, d, *J* = 2.2 Hz, H-6), 5.94 (1H, d, *J* = 2.2 Hz, H-8), 6.90 (1H, d, *J* = 8.6 Hz, H-3′), 7.39 (1H, d, *J* = 8.6 Hz, H-2′), 12.18 (1H, s, OH-5); EI-MS *m/z* (%): 272 (M^+, 100), 179 (24), 153 (80), 120 (48).

***trans*-3,4′,7-Trihydroxyflavanone (24)**

IR ν_{max}: 3271, 1669, 1610, 1608 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 4.41 (1H, d, *J* = 3.2 Hz, OH-3), 4.56 (1H, dd, *J* = 12.0, 3.2 Hz, H-3), 5.04 (1H, d, *J* = 12.0 Hz, H-2), 6.39 (1H, d, *J* = 2.4 Hz, H-8), 6.61 (1H, dd, *J* = 8.4, 2.4 Hz, H-6), 6.87 (1H, d, *J* = 8.8 Hz, H-2′), 7.42 (1H, d, *J* = 8.8 Hz, H-3′), 7.72 (1H, d, *J* = 8.4 Hz, H-5); EI-MS *m/z* (%): 272 (M^+, 20), 243 (75), 149 (32), 137 (100), 107 (45).

Vanillin (25)

IR ν_{max}: 3217, 1668, 1590, 1510 cm^{-1}; mp: 82-83 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.95 (3H, s, -OCH_3), 6.20 (1H, s, -OH), 6.99 (1H, d, *J* = 2.0 Hz, H-5), 7.38 (1H, d, *J* = 2.0 Hz, H-2), 7.40 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 9.81 (1H, s, -CHO); EI-MS *m/z* (%): 152 (M^+, 100), 123 (30), 109 (16).

Methyl ferulate (26)

IR ν_{max}: 3399, 1706, 1637, 1594, 1516 cm^{-1}; mp: 62-63 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.78 (3H, s, -$COOCH_3$), 3.90 (3H, s, -OCH_3), 5.89 (1H, s, -OH), 6.91 (1H, d, *J* = 8.1 Hz, H-5), 7.00 (1H, d, *J* = 1.9 Hz, H-2), 7.07 (1H, dd, *J* = 8.1, 1.9 Hz, H-6), 6.28 (1H, d, *J* = 15.9 Hz, H-7), 7.60 (1H, d, *J* = 15.9 Hz, H-8); EI-MS *m/z* (%): 208 (M^+, 100), 177 (68), 145 (36).

3,5-Dihydroxytoluene (27)

IR ν_{max}: 3356, 1604, 1480 cm^{-1}; mp: 108-110 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.21 (3H, s, -CH_3), 6.15 (1H, t, *J* = 2.4 Hz, H-4), 6.22 (2H, d, *J* = 2.4 Hz, H-2, H-6); EI-MS *m/z* (%): 124 (M^+, 100), 123 (78), 95 (20).

4-Hydroxy-3,5-dimethoxybenzaldehyde (28)

IR ν_{max}: 3415, 1684, 1609, 1514 cm^{-1}; mp: 114-116 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.96 (6H, s, -OCH_3), 6.05 (1H, s, -OH), 7.14 (2H, s, H-2, H-6), 9.80 (1H, s, -CHO); FAB-MS *m/z* (%): 183 (M^++1, 15), 154 (100), 136 (66).

Methyl gallate (29)

IR ν_{max}: 3442, 1700, 1614, 1540 cm^{-1}; mp: 240-242 °C; ^{1}H-NMR (CD_3COCD_3) δ: 3.78 (3H, s, -OCH_3), 7.10 (2H, s, H-2, H-6); EI-MS *m/z* (%): 184 (M^+, 52), 153 (100), 125 (26).

2,6-Dimethoxy[1,4]benzoquinone (30)

IR ν_{max}: 3415, 1697, 1646, 1596, 1447 cm^{-1}; mp: 255-258 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.80 (6H, s, -OCH_3), 5.84 (2H, s, H-2, H-6); FAB-MS *m/z* (%): 169 (M^++1, 15), 154 (100), 136 (69).

3,4,5-Trimethoxybenzyl alcohol (31)

IR ν_{max}: 3400, 1610, 1505 cm^{-1}; mp: 36-38 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.82 (3H, s, -OCH_3), 3.85 (6H, s, -OCH_3), 4.62 (2H, s, -CH_2OH), 6.59 (2H, s, H-2, H-6); FAB-MS *m/z* (%): 198 (M^+, 20), 154 (100), 137 (60), 136 (68).

Gallic acid (32)

IR ν_{max}: 3364, 3300-2600, 1682, 1616, 1455 cm^{-1}; mp: 258-260 °C; ^{1}H-NMR (CD_3OD) δ: 7.06 (2H, s, H-2, H-6); EI-MS *m/z* (%): 170 (M^+, 100), 153 (66).

Ficusol (33)

IR ν_{max}: 3419, 1731, 1602, 1518 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 3.69 (3H, s, -$COOCH_3$), 3.72 (1H, dd, *J* = 10.5, 5.3 Hz, H-2), 3.80 (1H, dd, *J* = 12.9, 5.3 Hz, H_b-3), 3.87 (3H, s, -OCH_3), 4.09 (1H, dd, *J* = 12.9, 10.5 Hz, H_a-3), 5.50 (1H, s, -OH), 6.72 (1H, dd, *J* = 8.6, 1.9 Hz, H-6′), 6.76 (1H, d, *J* = 1.9 Hz, H-2′), 6.85 (1H, d, *J* = 8.6 Hz, H-5′); EI-MS *m/z* (%): 226 (M^+, 100), 196 (96), 195 (94), 164 (60).

2-Hydroxy-6-pentadec-8(*Z*)-enylbenzoic acid (34)

IR ν_{max}: 3436, 2925, 2850, 1648, 1604 cm^{-1}; mp: 136-137 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, H-15′), 1.59 (2H, m, H-2′), 2.00 (4H, m, H-7′, H-10′), 2.96 (2H, t, *J* = 7.6 Hz, H-1′), 5.33 (2H, m, H-8′, H-9′), 6.74 (1H, d, *J* = 7.2 Hz, H-5),

6.84 (1H, d, J = 8.0 Hz, H-3), 7.31 (1H, dd, J = 8.0, 7.2 Hz, H-4), 8.34 (1H, brs, COOH-1), 11.01 (1H, brs, OH-2); EI-MS *m/z* (%): 364 (M^+, 8), 302 (12), 149 (16), 108 (100).

5-Formylmellein (35)

IR ν_{max}: 3121, 2710, 1673, 1578, 1476 cm^{-1}; mp: 127-129 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.58 (3H, d, J = 6.4 Hz), 3.05 (1H, dd, J = 18.0, 11.6 Hz, H_a-4), 3.95 (1H, dd, J = 18.0, 3.2 Hz, H_b-4), 4.72 (1H, m, H-3), 7.05 (1H, d, J = 8.8 Hz, H-7), 7.92 (1H, d, J = 8.8 Hz, H-6), 10.01 (1H, s, CHO-5), 11.94 (1H, brs, OH-8); EI-MS *m/z* (%): 206 (M^+, 96), 191 (100), 163 (75), 136 (68).

5-Hydroxymethylmellein (36)

IR ν_{max}: 3415, 1675, 1615, 1480 cm^{-1}; mp: 111-112 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.54 (3H, d, J = 6.4 Hz), 2.86 (1H, dd, J = 17.2, 9.0 Hz, H_a-4), 3.17 (1H, dd, J = 17.2, 3.2 Hz, H_b-4), 4.60 (2H, s, CH_2OH-5), 4.70 (1H, m, H_α-3), 6.87 (1H, d, J = 8.4 Hz, H-7), 7.43 (1H, d, J = 8.4 Hz, H-6), 11.16 (1H, s, OH-8); EI-MS *m/z* (%): 208 (M^+, 100), 190 (28), 178 (40), 161 (39).

Alkyls caffeate (37)

IR ν_{max}: 3420, 1690, 1602, 1464 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.85 (3H, t, J = 6.8 Hz), 1.65 (2H, m, H-2′), 4.16 (2H, t, J = 6.6 Hz, H-1′), 6.24 (1H, d, J = 16.0 Hz, H-8), 6.84 (1H, d, J = 8.2 Hz, H-5), 6.98 (1H, dd, J = 8.2, 2.0 Hz, H-6), 7.07 (1H, d, J = 2.0 Hz, H-2), 7.55 (1H, d, J = 16.0 Hz, H-7); EI-MS *m/z* (%): 572 (M^+, 4), 544 (M^+, 14), 516 (M^+, 60), 488 (M^+, 10), 180 (100), 163 (64).

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