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STUDIES ON THE CONSTITUENTS OF BULBS OF THE ORCHID *Prosthechea michuacana* AND ANTIOXIDANT ACTIVITY

Rosa Martha Perez Gutierrez,[1*] Adriana Maria Neira Gonzalez,[1] Efren Garcia Baez,[2] and Sandra Lugardo Diaz[1]

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Bioassay-guided fractionation of the chloroform extract of bulbs of the orchid P. michuacana *was used to determine the chemical identity of bioactive constituents. The use of DPPH assay led to the isolation of two new lanostane triterpenoids 3α-acetoxy, 24-hydroxy-24-methyl-5α-lanosta-9(11),25-diene (**1**) and 3α-acetoxy, 24-hydroxy-24-methyl-5α-lanosta-9(11)ene (**2**), a new stilbene α-α′-dihydro,3′,5′,2-trimethoxy-3-hydroxy-4-acetyl-4′-isopentenylstilbene (**3**), the phenanthrene 4,6,7-trihydroxy-2-methoxy-8-(methylbut-2-enyl)phenanthrene-1,1′-4′,6′,7′-trihydroxy-2′-methoxy-8′-(methylbut-2′-enyl)phenanthrene (**4**), one new abietane-type diterpene 12-hydroxy-3β,7β,18α-triacetoxy-8,11,13-abietatriene (**5**), together with gigantol (**6**), a known compound. The compounds were identified by spectral analysis and comparison with spectroscopic data reported in the literature. Compounds **3**, **4**, **5**, and **6** showed DPPH and ABTS radical-scavenging and anti-lipid peroxidation activities, but none of the isolated triterpenes showed promising antioxidant activity.*

Keywords: *Prosthechea michuacana,* antioxidant activity, culinary orchid.

Since pre-Hispanic times, orchids have been used in medicine in Mexico. They can be handmade and are edible; they are used for narcosis and as a favoring agent, poison, and glue; they are also used in foodstuffs and for ceremonial purposes, and in magical and religious talismans and aphrodisiacs [1]. Most orchids are known mainly for their ornamental value in America and Europe but not in Asia, where they have a long history in medicine. Mixtecos and Zapotecos of Oaxaca used as food the bulbs of *P. michuacana*, chew on the crude to quench thirst or liquefied it with a little water to prepare a drink; it is also used as an anti-inflammatory for the kidney, depurative for the circulatory system (diuretic), wound healing, and in the treatment of diabetes [2]. It is also used in the State of Oaxaca as an ornament to adorn the traditional "birth" in the months of November and December. Some biochemical studies of this orchid found that *P. michuacana* contains 8-*C*-(6-deoxy-*β*-D-glucopyranosyl)apigenine, 1-(3′-hydroxy-5′-methoxyphenyl)-2-(4′′-hydroxy-5′′-methoxyphenyl) ethane, and 2-(4-hydroxybenzyl)malic acid [3]. We reported in previous studies their relaxant and antispasmodic effect in isolated guinea pig ileum [4], their hepatoprotective properties, their inhibition of oxidative stress in liver [5], and their anti-inflammatory and wound healing activities [6]. The current investigation is an attempt to study the antioxidative activities of triterpenoids, stilbenes, phenolics, and abietane-type diterpenes from the bulbs of *Prosthechea michuacana.*

From the chloroform extract of the bulbs of the orchid, and after a series of extensive chromatographic separations, the fractionation of the crude extract followed by antioxidant TLC assay afforded **1**, **2**, **3**, **4**, **5** and **6**. The structural determination of compounds was based on their NMR spectra as well as on comparison with literature data for similar compounds.

1) Laboratorio de Investigacion de Productos Naturales, Escuela Superior de Ingenieria Quimica e Industrias extractivas IPN. Punto Fijo 16, Col. Torres Lindavista, cp 07708, Mexico D. F., e-mail: rmpg@prodigy.net.mx; 2) Departamento de Ciencias Basicas-Area de Quimica, Unidad Profesional Interdisciplinaria de Biotecnologia IPN. Av. Acueducto S/N, Barrio la laguna Ticoman, CP 07340. Mexico D. F., e-mail: efren1003@yahoo.com.mx. Published in Khimiya Prirodnykh Soedinenii, No. 4, pp. 469–474, July–August, 2010. Original article submitted March 29, 2009.

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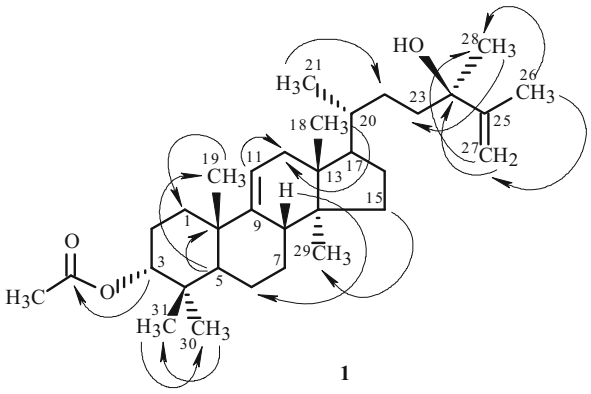

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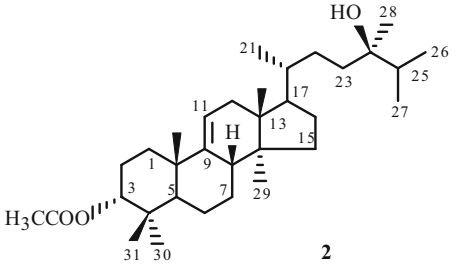
Fig. 1. H-C long-range correlations in the HMBC spectrum of **1**.

Compound **1** was obtained as an oil, and its molecular formula was determined as $C_{33}H_{54}O_3$ by HR-MS. The IR spectra of compound **1** showed absorption bands at 3427 cm^{-1} (OH), and one absorption band in the carbonyl region at 1719 cm^{-1} (acetate). Analysis of the ^{13}C and DEPT spectra revealed that it possesses nine methyls, ten methylenes, six methines, and eight quaternary carbons. In the NMR spectra the signals at δ_H 1.81 and δ_C 28.84 suggested the presence of the methyl group attached to a quaternary carbon (δ_C 74.01) bearing a hydroxyl group; a secondary methyl group was then placed at C-20 (δ_H 0.92 d, J = 6.4 Hz; δ_C 18.99); signals for five methyl groups attached to sp^3 carbon atoms at δ_H 1.81, 1.01, 0.78, 0.82, 0.84 were also found. The correlations observed for these five methyl proton signals clearly indicated a tetracyclic triterpene. The analyses of the ^1H and ^{13}C NMR spectra, including COSY and HMBC (Fig. 1), suggested the presence of a C-9 side chain containing three methyl group. The connectivity of the protons H-5 and H-8 and the methylene proton H_2-15 demonstrated that the compound has a lanostane skeleton [7]. The presence of an acetoxyl function attached to a cyclohexane ring in **1** was indicated by the appearance of a signal at δ_H 4.47 for a methine proton. The chemical shifts and the splitting patterns indicated their axial coupling nature, with an axial and an equatorial proton attached to one of the neighboring carbon atoms, the other adjacent carbon atom being fully substituted. The mass spectrum of **1** showed intense peaks at *m/z* 354 attributed to the ion-fragment formed by the loss of the C-17 side chain and two hydrogen atoms. Fragment ions at *m/z* 354, 325, and 296 showed one double bond in the side chain and the other in the ring system [8]. The peaks at *m/z* 457, 385, 369, and 324 indicated the placement of the hydroxyl group (C-24) and the double bond in the side chain at C-27 [9]. The ^1H spectra showed the presence of a methyl group connected with the double bond (broadened singlet at δ 1.61). Therefore, any other arrangement of the side chain was excluded. The ion at *m/z* 498 and 438 indicated the loss of a hydroxyl and acetoxyl group, respectively. The fragment ions at *m/z* 255, 241, and 229 are indicative of $\Delta^{9(11)}$ triterpenoids [10, 11], as confirmed by a tribustituted vinylic proton at δ_H 5.32, δ_C 114.72. The structure of **1** was finally confirmed by ^{13}C NMR spectral data [7] as 3α-acetoxy,24-hydroxy-24-methyl-5α-lanosta-9(11),25-diene.

A comparison of the ^1H and ^{13}C NMR spectra of **1** and **2** revealed that the spectra were only different with respect to the signals for C-25, C-26, and C-27. In compound **2** the signals at δ 0.80 and 0.82 were due to secondary methyls. The presence of the $C_9H_{17}O$ side chain was evident from the appearance of the ion M-139 in the spectra of **1**, which was displaced to M-141 in the spectrum of **2**, indicating that the side chain is saturated, as confirmed by the presence of an isopropyl group at δ_C 29.20, 19.40, and 19.10 in the ^{13}C NMR spectra. These results indicate that compound **2** is 3α-acetoxy,24-hydroxy-24-methyl-5α-lanosta-9(11)ene.



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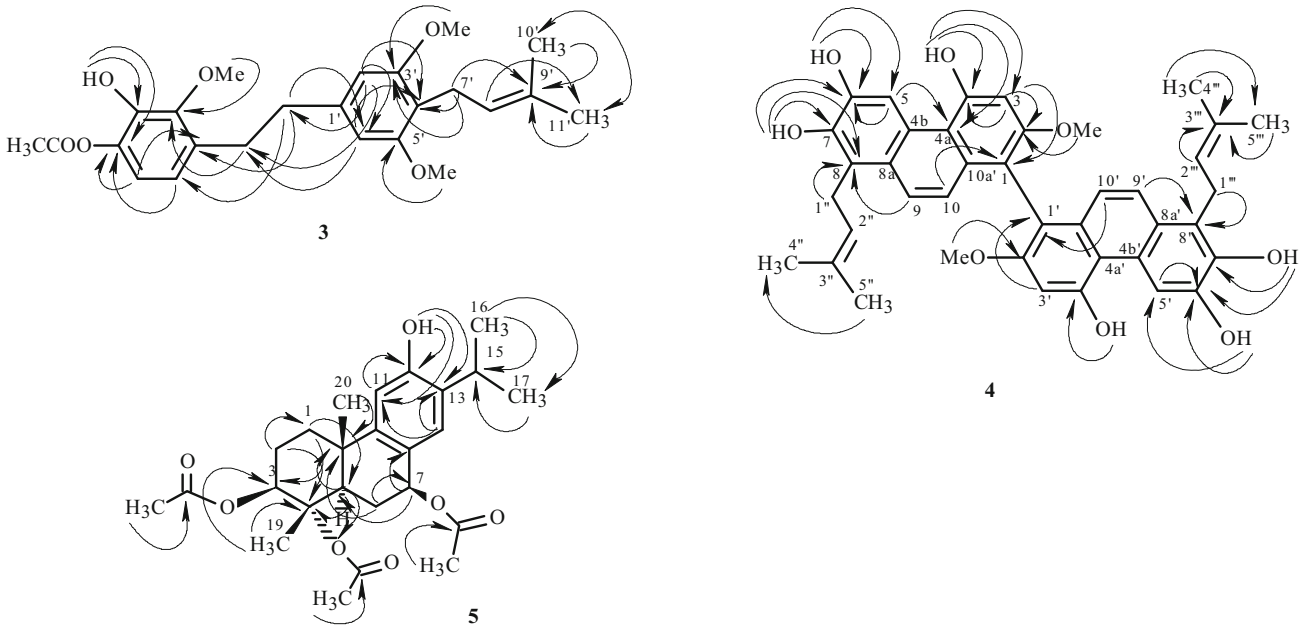

Fig. 2. H-C long-range correlations in the HMBC spectrum of **3–5**.

Compound **3** was obtained as a red oil. The IR spectrum showed absorptions at 3404 cm^{-1} (OH) and 1602 cm^{-1} (benzenoids), which indicated the phenolic nature of the compound. The HR-EI-MS, *m/z* 414.256 $[M + H]^+$, and NMR analyses revealed the molecular formula as $C_{24}H_{30}O_6$. The presence of the phenolic group in the structure was indicated by its characteristic color reaction with $FeCl_3$ (violet). Twenty-four carbon signals due to six methyl, three methylenes, five methines, and ten quaternary carbons were observed in the ^{13}C NMR and DEPT spectra. The analysis of the aromatic region of the ^1H NMR spectrum led us to establish the substitution pattern of the aromatic ring, in particular, the signal at δ 7.44 for one phenolic hydroxyl proton, four aromatic protons appearing as a two-proton singlet at δ 6.43 (2H, s), two neighboring protons at δ 6.81 (1H, dd, J = 2.1, 8.5 Hz, H-5) and δ 7.60 (1H, d, J = 8.5 Hz, H-6), three *O*-methyl groups at δ 3.82 (6H, s, OMe) and δ 3.93 (3H, s, OMe), and one acetyl at δ 2.11. The two methylene groups at δ 2.86 are typical of the ethane bridge in bibenzylic compounds [12]. According to the ^1H and ^{13}C data, a bibenzyl skeleton with one hydroxyl, one acetyl, one prenyl, and three *O*-methyl groups was deduced [13]. The signal at δ_C 112.4 is assigned to the nonprotonated carbon atom C-4′, based on correlations to the two-proton singlet at δ_H 6.43 (H-2′, H-6′) as well as due to the correlation of C-4′ to the two-proton doublet at δ_H 3.31 (H-7′); C-4′ was identified as the site of attachment of the prenyl group. One triplet partially overlapped at δ_H 5.30 is indicative of the presence of one vinyl proton (H-8′) attributable in the isopentenyl group. The placement of the two aromatic protons of ring A in the *ortho* position and of the phenolic substituents at C-3′, C-5′, C-2, C-3, and C-4 was supported by HMBC correlations, of which some of the more significant are shown in Fig. 2. The structure of **3** was determined as α-α′-dihydro,3′,5′,2-trimethoxy-3-hydroxy-4-acetyl-4′-isopentenylstilbene.

Compound **4** showed the molecular formula $C_{40}H_{38}O_8$ as established by HR-MS measurement. The IR spectrum showed absorptions at 3400 (OH), 1699, and 1458 cm^{-1} (aromatic ring). The degree of protonation of each carbon was determined by DEPT experiments, and the assignment of the carbon chemical shifts was made by comparison with the δ_C values of structurally related compounds [14, 15]. Since **4** contains a total of 20 shifts, each of these signals corresponds to double the number of protons given by the respective integral ratios. This suggested the symmetrical dimeric formulation. The ^1H NMR spectrum of **4** showed signals due to a pair of *ortho*-coupled aromatic protons (δ 6.95 and δ 7.21 each d, J = 9 Hz); they are assigned to H-9, H-9′ and H-10, H-10′, respectively, although these signals, particularly at δ_H 6.95, showed considerable upfield shift compared to those for H-9 and H-10 (~ δ 7.50) of monomeric phenanthrene derivatives [16]. Since the chemical shift of C-1 indicated that it was not oxygenated, it must be the point of attachment of the second phenanthryl unit, which supports a 1,1′-coupling of two monomeric halves in compound **4** [17]. The presence of three phenolic hydroxyl groups was indicated by the singlet at δ_H 7.62, 7.60, and 7.42 and two isolated aromatic protons (δ_H 9.94 and δ_H 6.31).

TABLE 1. Antioxidant Activity of Compounds **1–6**

Compound	DPPH	IC_{50}, μg/mL Anti-lipid peroxidation	ABTS
3	55 ± 0.41	47 ± 1.12	812 ± 1.40
4	7.5 ± 0.85	24 ± 0.98	523 ± 0.99
5	15.4 ± 0.13	29 ± 0.87	624 ± 2.31
6	23 ± 0.41	35 ± 1.16	704 ± 1.95
dl-α-Tocopherol	5.7 ± 0.13	20 ± 0.76	–
Quercetine	2.6 ± 0.27	13 ± 0.28	–
BHT	–	–	545 ± 1.02

Data were expressed as mean ± SD. Concentrations required to produced IC_{50} (μg/mL). (–) not tested.

The most downfield aromatic proton signal at δ 9.94 is typical of H-5 in a phenanthrene derivative [18]. If this signal is assigned to H-5, it implies that H-4 must be substituted, and the appearance of this signal as a sharp singlet further indicates that C-6 and C-7 are also substituted [19]. As shown in Fig. 2, the quaternary carbons at δ_C 159.34 showed long-range correlation with the methoxyl protons at δ 3.96 (OMe). Therefore, this was assigned unequivocally to C-2, and the remaining downfield carbons δ_C 145.34, 146.71, and 147.63 must be linked to hydroxyl groups. The location of the methoxyl, hydroxyl, and isopentenyl groups was determined from the HMBC spectrum. From the spectral data the structure of **4** was assigned as 4,6,7-trihydroxy-2-methoxy-8-(methylbut-2-enyl)phenanthrene-1,1′-4′,6′,7′-trihydroxy-2′-methoxy-8′-(methylbut-2′-enyl)phenanthrene.

Compound **5** has the molecular formula $C_{25}H_{34}O_7$. The IR spectrum showed absorption bands for acetoxy groups and a phenolic hydroxyl. The analysis of the ^{13}C and DEPT spectra revealed signals for five tertiary methyl groups, two secondary methyl groups, three methylenes, four methines, and six quaternary carbons. The abieta-8,11,13-triene carbon skeleton was suggested on the basis of ^{13}C NMR data and also by comparison with the literature [20–22]. The appearance of the aromatic proton as singlets at δ 7.90 (H-11) and δ 7.65 (H-14) suggested two substituents at C-12 and C-13. The ^1H NMR spectrum showed signals for one proton at δ 4.83 (dd, J = 11.0, 5.5 Hz), suggesting the presence of an equatorially disposed C-3-OAc. The correlations between H-3 and H-2β and Me-19 in the ^1H–^1H COSY spectrum confirm this position. The peak at δ 7.65 (1H, s) for the C-14 aromatic proton is deshielded by the acetyl group at C-7, as observed in similar diterpenoids [23]. The downfield shift of the 20-Me signal at δ 1.65 confirms the presence of an acetyl group at C-7. Examination of the ^1H NMR and 2D COSY spectra indicated the presence of an isopropyl group, evidently from two methyl doublets at δ 1.13 and 1.14 coupled to a methine group at δ 3.44, as confirmed by the ^{13}C NMR spectra (δ 26.7, 22.4, and δ 22.6). The position of the angular methyl group at C-20 was assigned by COLOC, which showed long-range coupling of δ_H 1.65 with δ_C 144.1 (C-9). The relative stereochemistry of the C-20-methyl group and H-5 was found to be *trans*. The close proximity of the protons represented by the methine signal at δ_H 7.90 (H-11) to the doublets at δ_H 1.70 (H-1α) and doublets at δ_H 4.83 (H-3_{ax}) was established by NOESY. Similarly, proximity of the isopropyl group to the aromatic methine at δ_H 7.90 (H-11) was observed. In the HMBC spectrum, methyl H-19 exhibited a ^2J correlation to C-4 and ^3J to C-3, C-5, and to the carbonyl carbon of C-18 (δ_C 173.1), indicating the presence of a methyl ester at C-18 [24]. The most important correlations of the HMBC study are shown in Fig. 2, leading to the structure 12-hydroxy-3β,7β,18α-triacetoxy-8,11,13-abietatriene.

The known compound **6** gigantol (3′,4-dihydroxy-3,5′-dimethoxybibenzyl) was identified by its IR, ^1H NMR, and ^{13}C NMR, which was previously isolated from *P. michuacana* and other orchids [4].

The concentration of total extractable phenolics in the orchid bulbs was 21.65 GAE/g of dry material. These results may suggest that although DPPH radical-scavenging activity might be an indicator of potential antioxidant activity, there may not always be a linear correlation between these two activities. The antioxidant activities of putative antioxidants have been attributed to various mechanisms; among these are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging [25, 26].

The well-known antioxidants vitamin E and quercetin were used as positive controls. In the DPPH assay, compounds **3**, **4**, **5**, and **6** exhibited scavenging activity. The results reveal that 4,6,7-trihydroxy-2-methoxy-8-(methylbut-2-enyl)phenanthrene-1,1′-4′,6′,7′-trihydroxy-2′-methoxy-8′-(methylbut-2′-enyl)phenanthrene possesses a significant inhibitory activity against DPPH radical, followed by 12-hydroxy-3β,7β,18α-triacetoxy-8,11,13-abietatriene and gigantol, with α-α′-dihydro,3′,5′,2-trimethoxy-3-hydroxy-4-acetyl-4′-isopentenylstilbene showing the least radical scavenging activity.

The results implied that the phenol structure is essential to its radical scavenging activity. Compound **4** displayed higher antioxidant activity than vitamin E because it posseses a structure bearing a catechol-like moiety on the B ring, confirming the greater protective effect of these polyphenols against lipid peroxidation [27].

The $FeCl_2$-ascorbic acid stimulated lipid peroxidation method is an indirect measure of lipid peroxidation, which is susceptible to interference by endogenous and exogenous substances; it should be regarded as an indication rather than as an absolute measure of total tissue lipid peroxide levels [28], possibly the mechanism by which compounds function as antioxidant in inhibiting iron-induced LDL oxidation. The phenolic hydroxyl groups provide hydrogen atoms to neutralize LDL lipid radicals; this mechanism is supported by our observation that compounds can react with the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), which accepts a hydrogen atom as it is neutralized [29]. On the other hand, it is possible that it exerts its antioxidant effect by chelating iron ions, a property shared by terrestrial plant phenolic antioxidants [30].

Compound **4** exhibited much stronger LDL anti-oxidation activity, like α-tocopherol. However, the isolated compounds **3**, **5**, and **6** are not as potent as α-tocopherol and quercetin. It has been reported that free OH groups in phenolic compounds are mainly responsible for antioxidant activity [31]. Compound **4** contains more hydroxyl groups than the other compounds isolated. This may also be the reason for the higher antioxidant activity.

Compounds **3**, **4**, **5**, and **6** were fast and effective scavengers of the ABTS radical (Table 1) and this activity was comparable to that of BHT. Proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm, which decreases with the scavenging of the proton radicals [32]. The scavenging of the $ABTS^+$ radical by **4** was found to be much higher than that of the DPPH radical. Factors like stereoselectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react and quench different radicals [33]. This further showed the capability of the compounds to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathological damage. We conclude that compounds **3–6** isolated form *P. michuacana* exhibit scavenging activity and anti-lipid peroxidation activities. This may be useful for the treatment of oxidative damage of tissues caused by the generation of reactive oxygen species.

EXPERIMENTAL

General Experimental Procedures. Melting points were measured without correction on an Electrothermal model 9100 instrument. IR spectra were obtained on a Perkin–Elmer FTRI 1720X instrument. A Bruker DRX-600 NMR spectrometer operating at 599.19 MHz for ^1H and 150.86 MHz for ^{13}C, using the UXNMR software package, was used for NMR experiments; chemical shifts are expressed in δ (ppm) using TMS as an internal standard. DEPT ^{13}C, ID COLOC, COSY, NOESY, and HMBC NMR experiments were carried out using the conventional pulse sequences as described in the literature. MS were measured on a JEOL HX 110 mass spectrometer. Column chromatography and preparative TLC were performed over silica gel (Merck) and Sephadex LH-20 (Pharmacia).

Plant Material. *Prosthechea michuacana* (Lex.) W. E. Higgins belongs to the family Orchidaceae. Bulbs were collected from El Punto, municipio de Santa Catarina Ixtepeji, distrito de Ixtlan, Oaxaca state, Mexico in May of 2007 and were taxonomically authenticated in the Herbario OAX and the Cassiano Conzatti Botanical Garden, Instituto Politecnico Nacional, and a voucher specimen of the plant is stored for reference (No. 6478).

Antioxidant Assay. **Total Phenolic Content**. A determination of total phenolic content, measured as gallic acid (GA) equivalents (mg kg^{-1}), was made with Folin-Ciocalteu phenol reagent [34]. One-milliliter samples were diluted to 25% of the original concentration with methanol, and 0.5 mL of Folin-Ciocalteu phenol reagent (2.0 N) and 3.0 mL of Na_2CO_3 (200 g L^{-1}) were added in the given order. The mixtures were vortexed and the reactions allowed to proceed for 15 min at room temperature. The mixtures were then diluted with 10 mL of deionized water and centrifuged for 5 min at 1250 g, and the absorbance was measured at 725 nm. Methanol was used as a control in place of the sample. Gallic acid equivalents were determined from a standard concentration curve. Results were expressed as milligrames of gallic acid equivalent per gram of dry weight (mg GAE/gdw).

Radical Scavenging Effect on DPPH Radical. MeOH extract of bulbs and rhizomes at various concentrations (0.5–10 μg/mL) were added to a solution of DPPH (30 μM) in MeOH (0.6 mL), and the reaction mixture (total volume 1.2 mL) was shaken vigorously. After keeping at room temperature for 30 min, the remaining DPPH was determined by colorimetry at 520 nm, and the radical-scavenging activity of each compound was expressed using the ratio of the absorption of DPPH (%) relative to the control DPPH solution (100%) in the absence of sample. The mean values were obtained from triplicate experiments [35].

Anti-Lipid Peroxidation Activity: Thiobarbituric Acid Test. The effect of the test components on lipid peroxidation was determined by the method described by Yoshiyuki et al. [36]. The reaction mixture contained liver homogenate, tris-HCl buffer (pH 7.2), ascorbic acid, $FeCl_2$, and various concentrations of the test components. After incubation, distilled water and thiobarbituric acid were added, and the mixture shaken vigorously. *n*-Butanol was added to extract the products of malondialdehyde, and the samples were measured at 532 nm [37].

ABTS Radical Scavenging Assay. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 mL ABTS solution with 60 mL methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Fresh ABTS solution was prepared for each assay. The compounds (1 mL) were allowed to react with 1 mL of the ABTS solution, and the absorbance was taken at 734 nm after 7 min using the spectrophotometer [38]. The ABTS scavenging capacity of the compounds was compared with that of BHT.

The percentage inhibition was calculated as ABTS radical scavenging activity (%) = $[(Abs_{control} - Abs_{sample})]/(Abs_{control})\cdot 100$, where $Abs_{control}$ is the absorbance of ABTS radical + methanol and Abs_{sample} is the absorbance of ABTS radical + sample/standard.

Statistical Analysis. The experimental results were expressed as means ± standard deviation (SD) of three replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA), and the differences between samples were determined by Duncan′s Multiple Range test using the Statistical Analysis System (SAS, 1999) program. P values < 0.05 were regarded as significant.

Extraction and Isolation. The dry powdered bulbs of *P. michuacana* (2 kg) were defatted three times with hexane, and the residual material was extracted (× 2) with chloroform consecutively. The extracts were evaporated under reduced pressure. The chloroform extract (yield 10.3%) was applied to a silica gel column, eluting with $CHCl_3$–EtOAc (7:1). The fractions were collected and combined based on their TLC profiles to produce nine pooled fractions, which were subjected to antioxidant tests. FI and FIII were the fractions that showed antioxidant activity. The FI active fraction was chromatographed on silica gel using $CHCl_3$–EtOAc gradient (12→1) to yield five secondary fractions (FI-1 to FI-5). The most active fractions were FI-3 and FI-5. Rechromatography of FI-3 using a Si gel (230–400 mesh) column and eluting with ethyl ether yielded only five fractions (FI-3-1 to FI-3-5). Fractions FI-3-3 and FI-3-5 showed antioxidant activity, which were individually subjected to further column chromatography eluting with $CHCl_3$–methanol (7:1) to obtain subfractions FI-3-3-1 to FI-3-3-4 and FI-3-5-1 to FI-3-5-6, respectively. Subfractions FI-3-3-2, FI-3-3-4, and FI-3-5-3 showed antioxidant effect. Active fractions were purified subjected to repeated column chromatography on Sephadex LH-20 (chloroform with increasing amounts of methanol) to obtain **1** (30 mg), **2** (37 mg), **3** (42 mg), and **4** (53 mg). Fraction FI-5 was subjected to separation using column chromatography over silica gel and eluted with a gradient of hexane–acetone–ethyl ether (4:1.5:1.0) to produce seven fractions (FI-5-1 to FI-5-7). Antioxidant testing indicated one active fraction (FI-5-3). The subfraction FI-5-3 was subjected to repeated column chromatography, first on silica gel (chloroform–acetone 7:0.5) and then on Sephadex LH-20 to obtain **5** (21 mg). Fraction FIII was subjected to silica gel column chromatography and eluted with ethyl ether–ethyl acetate (7:1). The fractions (40 mL each) were combined according to TLC monitoring into six portions (FIII-1-1 to FIII-1-6). Active fraction FIII-1-3 was purified further using mixtures of chloroform and ethyl ether and chloroform–acetone by silica gel column chromatography. Final purification of the active subfractions FIII-1-3-2 was achieved by preparative TLC on 1 mm thick silica gel plates eluted with hexane–chloroform–methanol (2:7:1) and visualised with UV at 254 nm. The following metabolite was obtained: **6** (29 mg).

3α-Acetoxy,24-hydroxy-24-methyl-5α-lanosta-9(11),25-diene (1). Oil; IR ($CHCl_3$, ν_{max}, cm^{-1}): 3427 (OH), 2956, 2914, 2844, 1719 (ester), 1602 (nonconjugated double bond), 1458, 839 (C-H stretching of trisubstituted double bond), 1384, 1217, 1053, 933, 769; HR-EI-MS, *m/z* $([M + H])^+$ 498.0889 calcd for $C_{33}H_{54}O_3$, 498.0892; EI-MS *m/z* (rel. int.) 498 (49), 483 [M^+ – Me], (65), 438 [M^+ – acetic acid] (10), 423 [M^+ – Me – acetic acid] (57), 457 [M^+ – part of side chain by C-24, C-25 cleavage] (56), 411 (41), 385 [M^+ – part of side chain by C-20, C-22 cleavage] (25), 369 [*m/z* 438 – part of side chain by C-23, C-24 cleavage]$^+$ (28), 354 [M^+ – side chain-2H] (100), 325 [*m/z* 385 – acetic acid]$^+$ (17), 324 [*m/z* 438 – part of side chain by C-20, C-22 cleavage – H]$^+$ (23), 296 [M^+ – side chain – acetic acid], 255 (6), 241 (5), 249 (11), 229 (8), 189 (21), 187 (15); ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm, J/Hz): 0.66 (3H, s, Me-18), 1.01 (3H, s, Me-19), 0.92 (3H, d, J = 6.4, Me-21), 1.61 (3H, s, Me-26), 1.81 (3H, s, Me-28), 0.78 (3H, s, Me-29), 0.82 (3H, s, Me-30), 0.84 (3H, s, Me-31), 4.64 (H, d, J = 2.5, H-27), 4.71 (H, ddd, J = 2.5, 1.4, 1.4, H-27), 4.47 (1H, dd, J = 4.3,11, H-3α), 5.32 (1H, d, J = 6.2, H-11), 2.28 (3H, s, OCO$\underline{CH_3}$), 3.50 (1H, m, OH-24); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 32.11 (C-1), 23.25 (C-2), 81.29 (C-3), 37.44 (C-4), 50.30 (C-5), 21.28 (C-6), 26.19 (C-7), 42.49 (C-8), 147.42 (C-9), 39.96 (C-10), 114.72 (C-11), 36.71 (C-12), 43.62 (C-13), 44.21

(C-14), 33.02 (C-15), 28.47 (C-16), 50.46 (C-17), 14.37 (C-18), 22.93 (C-19), 36.36 (C-20), 18.99 (C-21), 34.12 (C-22), 31.86 (C-23), 28.84 (C-24), 153.82 (C-25), 114.72 (C-26), 19.24 (C-27), 18.24 (C-29), 29.56 (C-30), 24.52 (C-31), 21.28 ($OCO\underline{CH_3}$), 170.78 ($O\underline{CO}CH_3$).

3α-Acetoxy,24-hydroxy-24-methyl-5α-lanosta-9(11)ene (2). Oil; IR ($CHCl_3$, ν_{max}, cm^{-1}): 3416 (OH), 2960, 2937, 2851, 1727 (ester), 1629, 1462, 1380, 1275, 1213, 1065, 956, 769; HR-EI-MS, *m/z* 500 $([M + H])^+$ calcd for $C_{33}H_{56}O_3$, 500.0767; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm, J/Hz): 1.54 (m, H-25), 0.80 (3H, d, J = 6.0, H-26), 0.82 (3H, d, J = 6.0, H-27); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 29.20 (C-25), 19.40 (C-26), 19.10 (C-27).

α-α'-Dihydro,3′,5′,2-trimethoxy-3-hydroxy-4-acetyl-4′-isopentenylstilbene (3). Red oil; IR ($CHCl_3$, ν_{max}, cm^{-1}): 3404 (OH), 1719 (ester), 1602 (benzenoids), 1466 (arom); HR-EI-MS, $([M + H])^+$ 414.256 calcd for $C_{24}H_{30}O_6$, 414.131; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm, J/Hz): 6.81 (1H, dd, J = 2.1, 8.5, H-5), 7.60 (1H, d, J = 8.5, H-6), 6.43 (2H, s, H-2′ and H-6′), 2.86 (4H, m, H_2-α and H_2-α'), 1.81 (6H, br.s, Me-10′ and Me-11′), 3.31 (2H, d, J = 7.0, H-7′), 5.30 (1H, tq, J = 7.0, 1.0, H-8′), 7.44 (1H, s, OH-4), 3.82 (6H, s, OMe), 3.93 (3H, s, OMe), 2.11 (3H, s, $OCO\underline{CH_3}$); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 135.1 (C-1), 146.6 (C-2), 145.4 (C-3), 143.2 (C-4), 129.4 (C-5), 107.6 (C-6),133.7 (C-1′), 105.4 (C-2′), 147.3 (C-3′), 112.4 (C-4′), 147.3 (C-5′), 105.1 (C-6′), 22.6 (C-7′), 121.9 (C-8′), 136.1 (C-9′), 18.2 (C-10′), 25.7 (C-11′), 55.1 (OMe-3′,5′), 56.4 (OMe-2), 22.16 ($OCO\underline{CH_3}$), 171.12 ($O\underline{CO}CH_3$).

4,6,7-Trihydroxy-2-methoxy-8-(methylbut-2-enyl)phenanthrene-1-1′-4′,6′,7′-trihydroxy-2′-methoxy-8′-(methylbut-2′-enyl)phenanthrene (4). Oil; IR ($CHCl_3$, ν_{max}, cm^{-1}): 3400 (OH), 1699, 1598, 1458 (arom); HR-EI-MS, $([M + H])^+$ 646.256 calcd for $C_{40}H_{38}O_8$, 646.131; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm, J/Hz): 9.94 (2H, s, H-5, H-5′), 6.31 (1H, d, J = 2.4, H-3, H-3′), 6.95 (1H, d, J = 9, H-9 and H-9′), 7.21 (1H, d, J = 9, H-10 and H-10′), 3.46 (2H, d, J = 6.7, H-1″ and 1‴), 5.46 (1H, tqq, J = 6.7, 1.5, H-2″ and 2‴), 1.61 (3H, s, H-4″ and 4‴), 1.26 (3H, d, J = 1.5, H-5″ and 5‴), 7.62 (1H, br.s, OH), 7.60 (1H, br.s, OH), 7.42 (1H, br.s, OH), 3.96 (3H, s, OMe). ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 101.31 (C-1, C-1′), 159.34 (C-2, C-2′), 100.08 (C-3, C-3′), 145.34 (C-4, C-4′), 117.2 (C-4a, C-4a′), 126.63 (C-4b), 123.18 (C-5, C-5′), 147.63 (C-6, C-6′), 146.71 (C-7, C-7′), 124.72 (C-8, C-8′), 136.94 (C-8a, C-8a′), 126.21 (C-9, C-9′), 127.89 (C-10, C-10′), 138.21 (C-10a, C-10a′), 25.42 (C-1″,C-1‴), 123.12 (C-2″,C-2‴), 134.25 (C-3″,C-3‴), 25.90 (C-4″, C-4‴), 18.64 (C-5″, C-5‴), 55.32 (OMe).

12-Hydroxy-3β,7β,18α-triacetoxy-8,11,13-abietatriene (5). Mp 185°C; IR ($CHCl_3$, ν_{max}, cm^{-1}): 3530 (OH), 3360–2700 (phenolic OH), 1745, 1255, 1235 (OAc), 1458 (arom); HR-EI-MS, $(M - H)^+$ 445.1255 calcd for $C_{25}H_{34}O_7$, 445.298; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm, J/Hz): 1.70 (1H, ddd, J = 14.0, 14.0, 5.0, H-1_{ax}), 3.43 (1H, ddd, J = 14.0, 3.5, 3.5, H-1_{eq}), 2.15 (1H, m, H-2), 4.83 (1H, dd, J = 11.0, 5.5, H-3_{ax}), 2.25 (1H, dd, J = 12.5, 2.0, H-5α), 1.53 (1H, m, H-6α), 1.81 (1H, m, H-6β), 4.15 (1H, br.d, J = 3.5, H-7), 7.90 (1H, s, H-11), 7.65 (1H, s, H-14), 3.44 (1H, septet, J = 7.0, H-15), 1.14 (3H, d, J = 4.3, H-16), 1.13 (3H, d, J = 4.3, H-17), 1.27 (3H, s, H-19), 1.65 (3H, s, H-20), 2.0 (s, OAc), 2.08 (s, OAc), 2.11 (s, OAc), 5.06 (br.s, OH). ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 30.8 (C-1), 22.9 (C-2), 79.5 (C-3), 43.6 (C-4), 35.9 (C-5), 30.1 (C-6), 67.2 (C-7), 128.2 (C-8), 144.1 (C-9), 41.5 (C-10), 112.6 (C-11), 151.7 (C-12), 132.4 (C-13), 126.3 (C-14), 26.7 (C-15), 22.4 (C-16), 22.6 (C-17), 27.8 (C-19), 24.9 (C-20), 170.4 ($O\underline{CO}CH_3$), 173.1 ($O\underline{CO}CH_3$), 175.3 ($O\underline{CO}CH_3$), 20.7 ($OCO\underline{CH_3}$), 21.4 ($OCO\underline{CH_3}$), 21.0 ($OCO\underline{CH_3}$).

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