A Trihomoabietane Diterpenoid from *Plectranthus grandidentatus* and an Unusual Addition of Acetone to the *ortho*-Quinone System of Cryptotanshinone

Cristina Gaspar-Marques,† M. Fátima Simões,† and Benjamín Rodríguez*,‡

Faculdade de Farmácia da Universidade de Lisboa, Centro de Estudos de Ciências Farmacêuticas (CECF), Avenida Prof. Gama Pinto, 1649-003 Lisboa, Portugal, and Instituto de Química Orgánica, Consejo Superior de Investigaciones Científicas (CSIC), Juan de la Cierva 3, E-28006 Madrid, Spain

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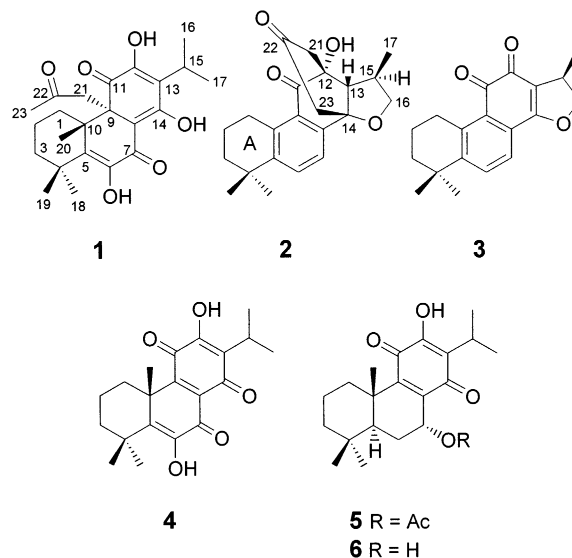
A new 9α-(2-oxopropyl)abietane derivative (**1**) has been isolated from an acetone extract of *Plectranthus grandidentatus*. Extraction of the plant material and analytical processes carried out in the absence of acetone also revealed the presence of **1** in the plant, thus suggesting that it is a natural product rather than an artifact. Attempts at obtaining Michael adducts between acetone and *para*-quinone abietane diterpenoids were unsuccessful, whereas treatment of the *ortho*-quinone cryptotanshinone (**3**) with acetone under basic conditions yielded compound **2**. The structures of **1** and **2** were established by 1D and 2D NMR spectroscopic studies.

Several highly functionalized abietane diterpenoids isolated from plants belonging to the *Plectranthus* and *Coleus* genera[1−3] (Labiatae) are substances of pharmacological interest. Abietane diterpenes have previously been reported from *Plectranthus grandidentatus* Gürke.[4] Recently, we have isolated other abietane diterpenoids from this plant and described their antibacterial, antifungal, and antitumor properties[5,6] and their inhibition of the proliferation of human lymphocytes induced by phytohaemagglutinin (PHA) mitogen.[7] Our desire to obtain additional quantities of these compounds to perform other biological assays required a new extraction of *P. grandidentatus.* We now wish to report on the structure elucidation of a new naturally occurring 2-oxopropylabietane derivative (**1**) isolated from this plant extract. In addition, we report an unusual synthetic compound (**2**) obtained by the reaction of the 20-nor-abietane cryptotanshinone (**3**)[8,9] with acetone under basic conditions.

Repeated chromatographic processes on an acetone extract of *P. grandidentatus* aerial parts (see Experimental Section) led to compound **1** together with the previously known abietane diterpenoids 7α-acetoxy-6β-hydroxyroyleanone[5,10−12] and coleon U[6,11−13] and a mixture of fatty acid esters of tyrosol.[14]

Combustion analysis and low-resolution mass spectrometry indicated the molecular formula $C_{23}H_{30}O_6$ for **1**, and its IR spectrum showed hydroxyl (3412, 3325 cm^{-1}), saturated ketone (1724 cm^{-1}), and α,β-unsaturated ketone (1652, 1561 cm^{-1}) absorptions. The ^1H and ^{13}C NMR spectra of **1** closely resemble those of coleon U-quinone (**4**), an abietane diterpenoid previously isolated[12] from the red leaf-glands of *Plectranthus argentatus* S. T. Blake. The observed differences between the ^1H and ^{13}C NMR spectra of **1** and **4** were consistent with the presence in the former of a hydrogen-bonded pseudoaromatic hydroxyl group [δ_{OH} 14.59, 1H, s; δ_C 171.5 (C)], instead of one of the carbonyl *p*-quinone carbons (δ_C 184.0)[12] of the latter, and with the presence in **1** of a 2-oxopropyl substituent [δ_H 3.37 and 3.23 (1H each, both d, J_{gem} = 16.7 Hz) and 1.94 (3H, s); δ_C 204.9 (C), 52.9 (CH_2), and 30.1 (CH_3)] attached to an sp^3 quaternary carbon (δ 53.8, C) of the abietane framework. In the HMBC spectrum of **1**, the carbonyl carbon of the 2-oxopropyl substituent showed connectivities with its vicinal Me-23 and 21-methylene protons, whereas both 21-methylene protons were connected with four fully substituted carbon atoms appearing at δ 106.2, 53.8, 46.3, and 196.4, which must be assigned to the C-8, C-9, C-10, and C-11 carbons, respectively, because these carbons showed HMBC cross-peaks with HO-14, Me-20, H_2-1, H_2-2, and Me-20 and with the HO-12, respectively. These connectivities support that the 2-oxopropyl substituent of **1** is attached to the C-9 position. This conclusion is also in agreement with the downfield shifts observed for the C-10 and C-11 carbons and with the diamagnetic shifts of C-5, C-8, C-9, C-14, and C-20 of **1** with respect to those of **4**[12] [$\Delta\delta = \delta(\mathbf{1}) - \delta(\mathbf{4})$: +5.1, +13.0, −3.1, −20.4, −101.1, −12.5, and −3.3 ppm, respectively]. The almost identical chemical shifts for C-1−C-4, C-6, C-7, C-12, C-13, and C-15−C-19 in **1** and **4**,[12] together with the remaining connectivities observed in the HMBC spectrum of **1** (those of C-5 with H_2-1, H_2-3, HO-6, Me-18, Me-19, and Me-20, those of H-15 with C-12−C-14, C-16, and C-17, and those of Me-20 with C-1, C-5, C-9, and C-10), further support structure **1** assigned to this new diterpenoid.



* Corresponding author. Tel: +34 915622900. Fax: +34 915644853. E-Mail: iqor107@iqog.csic.es.
† Faculdade de Farmácia da Universidade de Lisboa, CECF.
‡ Instituto de Química Orgánica, CSIC.

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A 9α-configuration for the 2-oxopropyl group of **1** was established by NOE experiments. Irradiation at δ 3.23 (H-21b proton) caused NOE enhancements only in its geminal (H-21a) and Me-23 proton signals, whereas only the signals of H-1β, H-2β, and Me-19 (δ 1.78, 1.64, and 1.22, respectively) were enhanced when Me-20 (δ 0.93) was irradiated. These results indicated that the Me-20 and 2-oxopropyl groups are on opposite sides of the plane of the molecule. The absolute configuration of **1** could not be determined. However, on biogenetic grounds we suppose that it belongs to the *normal* series, like coleon U-quinone (**4**)[12] and other abietane diterpenoids found in *Plectranthus* species.[1–4,10–13]

Since acetone was used for the extraction of the plant material, and structure **1** could be considered as a Michael adduct arising from a molecule of acetone and coleon U-quinone (**4**), **1** could be an artifact. This hypothesis was reinforced by the fact that the C-9 carbon of **4** (δ 154.9)[12] is the most deshielded of its olefinic carbons and consequently the choice site for an attack of a nucleophilic carbon of acetone. The 9α-configuration of the 2-oxopropyl group in **1** further supports artifact formation, since the addition of acetone to form **1** must occur from the less hindered α side of **4**. To determine if **1** is an artifact, another sample of the aerial parts of *P. grandidentatus* (with the same origin that the plant material used for the initial extraction) was extracted using $CHCl_3$ without acetone as solvent. Subsequent isolation procedures leading to **1** were carried out in the absence of acetone (see Experimental Section), and **1** was ultimately identified among the constituents of the extract. The appearance of **1** in the acetone-free extract provides evidence to support **1** as a natural compound in *P. grandidentatus*.[15]

Trihomoabietane diterpenoids are rare as natural products,[1–3] and only a few examples have been reported.[16–22] Some of these substances, such as danshenols A and B found in *Salvia miltiorhiza* Bunge[18] and *S. glutinosa* L.[19,20] (Labiatae), possess a 2-oxopropyl side chain attached to the C-11 position of a 19,20-dinor- or 20-nor-abietane hydrocarbon skeleton, respectively, and they have shown to be substances of natural origin.[18]

To investigate the plausible Michael-type addition of acetone to abietane quinone derivatives, we investigated this reaction with two diterpenoid benzoquinones that were available from previous studies.[9,23] Treatment of the *p*-benzoquinone 7α-acetoxyroyleanone[23–26] (**5**) with acetone under strong basic conditions (see Experimental Section) did not produce Michael adducts, whereas identical treatment of the *o*-benzoquinone cryptotanshinone[8,9] (**3**, $C_{19}H_{20}O_3$) gave a compound (**2**) whose molecular formula ($C_{22}H_{26}O_4$) indicated that an addition of acetone occurred. The structure of **2** was established as follows.

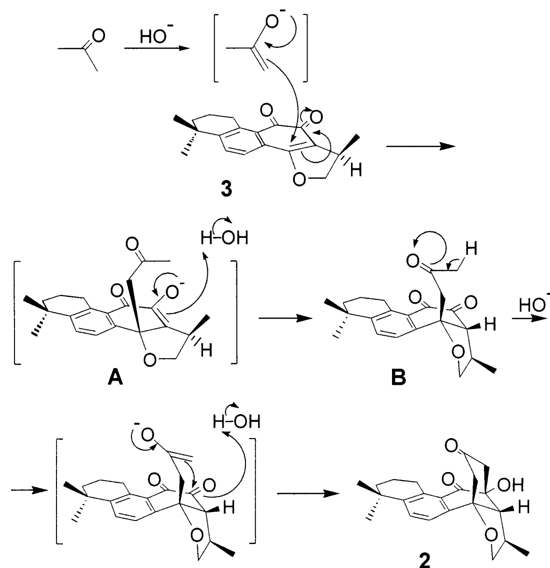
The UV spectrum of **2** showed characteristic absorptions for a substituted acetophenone chromophore[27] [λ_{max} (log ε) 215 (4.43), 258 (4.07), 308 (3.51) nm], and its IR spectrum indicated the presence of hydroxyl (3424 cm^{-1}), saturated ketone (1721 cm^{-1}), and aryl ketone (3101, 1677, 1590 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **2** drastically differed from those of cryptotanshinone[28,29] (**3**), although both compounds showed identical signals and similar chemical shifts for H_2-1–H_2-3, H_2-16 methylene, H-6, H-15 methine, and Me-17–Me-19 methyl groups, and for C-1–C-11 and C-15–C-19. The most significant differences between the ^1H and ^{13}C NMR spectra of **2** and **3** were consistent with the presence in the former of an additional 2-oxopropan-1,3-diyl part [δ_H 2.77 and 2.53 dd, J_{gem} = 17.0 Hz, $J_{long\ range}$ = 1.5 Hz, H_2-21, and δ 2.92 and 2.86 dd, J_{gem} = 14.5 Hz, $J_{long\ range}$ = 1.5 Hz, H_2-23; δ_C 52.6 (CH_2, C-21), 204.5 (C, C-22), and 54.8 (CH_2, C-23)] attached to two different quaternary carbons. In addition, C-12, C-13, and C-14 are transformed from three aromatic quaternary carbons in **3**[28,29] to two alkyl quaternary and a tertiary carbon in **2** [δ 76.1 (C, C-12), 61.6 (CH, C-13), and 82.3 (C, C-14)]. The HMBC spectrum of **2** showed two- and three-bond connectivities between both H_2-21 methylene and C-11–C-13, C-22, and C-23. On the other hand, C-8 (δ 145.0), C-13, C-14, C-21, and C-22 were connected with H_2-23. Moreover, the methine carbon at δ 61.6 (C-13) showed HMBC cross-peaks with HO-12, H-16a, and Me-17, and the sp^3 quaternary C-14 (δ 82.3) was connected with H-7, H-13, and H_2-16. In addition, the hydroxyl proton of **2** (δ 4.63 s) showed connectivities with the carbonyl carbon of the aryl ketone (δ 199.4) and C-12, C-13, and C-21. All these results firmly established that the 2-oxopropan-1,3-diyl substituent bridges between the C-12 and C-14 positions and that, apart from the configuration, this compound possesses structure **2**.

Since the absolute configuration of the C-15 asymmetric center of **3** is known,[8] a series of NOE experiments established not only the relative configuration of the 2-oxopropan-1,3-diyl bridge but also the absolute configuration of **2**. Irradiation at δ 2.62 (H-13β) produced NOE enhancements in the signals of HO-12α, H-16β, H-21a, and H-23a, as well as in those of H-15α and Me-17 (+1.4 and +4.1% NOE enhancement, respectively), whereas the signals of HO-12α, H-13β, H-21b, and H-23a were enhanced when H-21a (δ 2.77) was irradiated, thus establishing that all these protons are in close proximity of each other. Moreover, irradiation at H-15α (δ 2.05) caused NOEs only of HO-12α, H-16α (+2.9%), H-16β (+0.6%), and Me-17, and not of H-13β, thus confirming that H-13β and H-15α are on opposite sides of the tetrahydrofuran ring of **2**. Finally, irradiation at δ 1.21 (where both Me-17 and one of the C-4 methyl groups resonate) produced NOEs of H-13β, H-15α, and H-16β, together with those observed in H-6, H_2-3, and C-4 methyl (at δ 1.28), which were caused by the irradiation of the C-4 methyl substituent (δ 1.22). All these NOE experiments established that Me-17 and the C-21–C-23 bridge of **2** are *cis* oriented and that the orientation of H-13 is β.

A chair ($^{13}C_{22}$) conformation was established for the six-membered ring involving C-12–C-14 and C-21–C-23 of **2**. H-21a and H-23a (δ 2.77 and 2.92, respectively), which are close to H-13β (see above), appeared as doublets (J_{gem} = 17.0 and 14.5 Hz, respectively) in the ^1H NMR spectrum of **2**, whereas their methylene partners (δ 2.53 and 2.86, respectively) resonated as double doublets, showing a reciprocal long-range coupling (^4J = 1.5 Hz), which is typical for 1,3-diequatorial protons coupled through a carbonyl group.[30] These data support a chair ($^{13}C_{22}$) conformation and establish that H-21a and H-23a (pro-*S* and pro-*R* hydrogens, respectively) are axially oriented and H-21b and H-23b (pro-*R* and pro-*S* hydrogens, respectively) possess an equatorial orientation. Moreover, NOE experiments were also in agreement with these conclusions because irradiation at δ 2.77 (axial H-21a) produced, among others, NOEs in the H-23a and H-13β 1,3-diaxial protons, and irradiation at δ 2.53 (equatorial H-21b) did not cause NOEs in any of the 23-methylene protons.

It was not possible to establish the configurations of the C-1–C-3 methylene protons of **2** because H-1b, H_2-2, and H_2-3 overlapped. The observed vicinal coupling constants for the pseudoaxial H-1a proton (δ 3.19, $J_{a,a'}$ = 9.8 Hz, $J_{a,e'}$ = 5.8 Hz) are compatible with two possible half-chair ($^2HC_3$ and $^3HC_2$) conformations for ring A. In a $^2HC_3$ conforma-

Scheme 1. Formation of Compound **2** from Cryptotanshinone (**3**)



tion, the signal at δ 3.19 must be assigned to the pseudoaxial proton possessing an α-orientation, whereas that signal must be ascribed to the pseudoaxial 1β-proton when ring A possesses a $^{3}HC_{2}$ conformation. This ambiguity also precludes the assignment of the methyl groups attached to the C-4 carbon (Me-18 and Me-19: δ_H 1.28 and 1.22, δ_C 31.85 and 31.87). It seems that ring A of **2** does not possess a half-boat ($^{1,4}HB$ or $HB_{1,4}$) conformation, because no NOE was observed in any of the signals of the Me-18 and Me-19 groups when the pseudoaxial H-1a proton was irradiated. In any case, the possibility of a conformational equilibrium between $^{2}HC_{3}$ and $^{3}HC_{2}$ conformations in ring A of **2** could not be discarded.[31]

The transformation of cryptotanshinone (**3**) into **2** under basic conditions may be rationalized by the mechanistic pathway shown in Scheme 1, which implies a Robinson annelation reaction. A stereoselective attack of a carbanion arising from acetone on the C-14 position of **3** could produce the intermediate A, which is stereoselectively transformed into the Michael adduct B. Finally, an intramolecular cyclization of B affords **2** by an aldol condensation reaction between a carbanion at C-21 and the C-12 carbonyl group.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. UV spectra were recorded on a Perkin-Elmer Lambda 2 UV/vis spectrophotometer. IR spectra were obtained on a Perkin-Elmer Spectrum One spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on a Varian INOVA 400 apparatus at 400 and 100 MHz, respectively. Chemical shifts are given on the δ scale and were referenced to residual $CHCl_3$ at 7.25 ppm for proton and to the solvent at 77.00 ppm for carbon. All the assignments for protons and carbons were in agreement with 2D COSY, TOCSY, gHSQC, gHMBC, and 1D NOESY spectra. Mass spectra were registered in the positive EI mode on a Hewlett-Packard 5973 instrument (70 eV). Elemental analyses were conducted on a Carlo Erba EA 1108 apparatus. Merck Si gel (70−230 mesh and 230−400 mesh, for gravity flow and flash chromatography, respectively) was used for column chromatography. Merck 5554 Kieselgel 60 F_{254} sheets were used for TLC analysis. Petroleum ether (bp 50−70 °C) was used for column chromatography. HPLC analyses were performed on an apparatus equipped with a Merck Hitachi Lachrom 7100 pump, a Merck Hitachi D7500 integrator, and a Spectro-Physics Spectro-Chrom 100 UV detector (λ 432 nm). A Merck Lichrocart (125 × 4 mm) column with Lichrospher 100 RP-18 (5 μm) as stationary phase and 9:1 acetonitrile−water as mobile phase (flow rate 1 mL/min) was used.

Plant Material. *P. grandidentatus* Gürke was cultivated in the Faculty of Pharmacy Hortum, Lisbon University, from seeds provided by the National Botanic Garden, Kirstenbosh, Claremont, South Africa. The plants were cultivated through vegetative propagation of the first specimen obtained from seed. The material was collected in July−October 1997, and voucher specimens were deposited in the Herbarium of the Botanical Center of the "Instituto de Investigação Científica Tropical", Lisbon (ref. C. Marques, S/No. LISC).

Extraction and Isolation. Dried and powdered *P. grandidentatus* aerial parts (6.91 kg) were extracted with Me_2CO (3 × 25 L) at room temperature for 8 days. The solvent was evaporated under reduced pressure and low temperature (40 °C), yielding a residue (306.7 g). A part (150 g) of the total extract was subjected to column chromatography (Si gel 70−230 mesh, 1.5 kg) eluting successively with petroleum ether, petroleum ether−EtOAc (9:1, 4:1, 2:1, 1:1, and 1:4), EtOAc, 1:1 EtOAc−MeOH, and MeOH. The residue (9.26 g) of the fractions eluted with 9:1 petroleum ether−EtOAc was crystallized from 19:1 petroleum ether−EtOAc, giving pure 7α-acetoxy-6β-hydroxyroyleanone[5,10−12] (7α-acetoxy-6β,12-dihydroxyabieta-8,12-diene-11,14-dione, 3.32 g, 0.098% on dry plant material). The residue (5.72 g) obtained after evaporation of the mother liquors from the crystallization was rechromatographed (Si gel 230−400 mesh column, 340 g, eluted with a petroleum ether−CH_2Cl_2 gradient from 12:1 to 4:1), yielding, in order of increasing chromatographic polarity, a mixture of fatty acid esters of tyrosol[14] [2-(4-hydroxyphenyl)ethyl *n*-alkanoates, 32 mg, 0.0009%], impure **1** (87 mg), additional quantities of 7α-acetoxy-6β-hydroxyroyleanone (1.43 g, 0.042%), and coleon U[6,11−13] (6,11,12,14-tetrahydroxyabieta-5,8,11,13-tetraen-7-one, 7 mg, 0.0002%). Repeated crystallizations of impure **1** from *n*-pentane furnished a pure sample of **1** (25.6 mg, 0.0007%).

The previously known compounds (fatty acid esters of tyrosol,[14] 7α-acetoxy-6β-hydroxyroyleanone,[5,10−12] and coleon U[6,11−13]) were identified by their physical (mp, $[\alpha]_D$) and spectroscopic (^{1}H NMR, MS) data and by comparison (TLC) with authentic samples.

6,12,14-Trihydroxy-9α-(2-oxopropyl)abieta-5,8(14),12-triene-7,11-dione (1): yellow fine needles (*n*-pentane); mp 169−171 °C; $[\alpha]^{25}_{D}$ −694.6° (*c* 0.168, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 234 (3.90), 276 (3.99), 316 (3.96), 434 (3.95) nm; IR (KBr) ν_{max} 3412, 3325, 2961, 2927, 1724, 1652, 1561, 1397, 1359, 1293, 1211, 1146, 1037, 871, 774, 702, 654 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 14.59 (1H, s, HO-14), 7.39 (1H, s, HO-12), 6.62 (1H, s, HO-6), 3.39 (1H, sept, J = 7.1 Hz, H-15), 3.37 (1H, d, J_{gem} = 16.7 Hz, H-21a), 3.23 (1H, d, J_{gem} = 16.7 Hz, H-21b), 1.94 (3H, s, Me-23), 1.78 (2H, m,* H-1α and H-1β),[32] 1.64 (1H, m,* H-2β), 1.56 (1H, m,* H-2α), 1.55 (1H, m,* H-3β), 1.45 (3H, s, Me-18), 1.43 (1H, ddd, $J_{3\alpha,3\beta}$ = 14.0 Hz, $J_{3\alpha,2\alpha}$ = 2.4 Hz, $J_{3\alpha,2\beta}$ = 10.8 Hz, H-3α), 1.34 (3H, d, J = 7.1 Hz, Me-16 or Me-17), 1.33 (3H, d, J = 7.1 Hz, Me-17 or Me-16), 1.22 (3H, s, Me-19), 0.93 (3H, s, Me-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.9 (C, C-22), 196.4 (C, C-11), 179.3 (C, C-7), 171.5 (C, C-14), 152.7 (C, C-12), 143.2 (C, C-6), 139.9 (C, C-5), 127.9 (C, C-13), 106.2 (C, C-8), 53.8 (C, C-9), 52.9 (CH_2, C-21), 46.3 (C, C-10), 41.0 (CH_2, C-3), 35.8 (C, C-4), 31.1 (CH_2, C-1), 30.3 (CH_3, C-18), 30.1 (CH_3, C-23), 27.4 (CH_3, C-19), 24.9 (CH, C-15), 24.0 (CH_3, C-20), 19.7 (CH_3, C-16 or C-17), 19.3 (CH_3, C-17 or C-16), 17.6 (CH_2, C-2); EIMS *m/z* 402 $[M]^+$ (8), 387 (1), 384 (1), 369 (1), 359 (1), 345 (1), 251 (85), 233 (4), 223 (22), 151 (100), 123 (21), 111 (5), 91 (4), 81 (11), 69 (5), 67 (4), 55 (5), 43 (14), 41 (5); *anal.* C 68.71%, H 7.60%, calcd for $C_{23}H_{30}O_6$, C 68.63%, H 7.51%.

Identification of Compound 1 in a New Plant Extract by HPLC. *P. grandidentatus* aerial parts (131.5 g, with the same origin as the material used for the extraction with Me_2-

CO) were extracted with $CHCl_3$ for spectroscopy (Merck ref. 1.02447, 3 × 300 mL) at room temperature for 8 days. Filtration and evaporation (470 mbar, 40 °C) of the solvent gave a residue (3.07 g). A part (1.5 g) of this extract was subjected to chromatography (Si gel 230−400 mesh column, 80 g) eluting with petroleum ether (PRA grade, 99.5% C_6 isomers, free of Me_2CO) and petroleum ether−EtOAc (CHROMASOLV Plus, for HLPC, 99.9%, free of acetone) 9:1, 4:1, and 1:1 mixtures. The fractions eluted with 4:1 petroleum ether−EtOAc contained compounds with polarities similar (TLC) to that of **1**. Alternately, the remaining sample (1.57 g) of the total extract was digested with 9:1 petroleum ether−EtOAc (30 mL) at room temperature for 5 h. The soluble fraction was very similar (TLC) to that obtained by chromatography. Both these fractions were subjected to HPLC analysis (see General Experimental Procedures), and in both cases a peak with a retention time 2.5 min (identical to that of an authentic sample of **1**) was observed. HPLC of a mixture of authentic **1** and each one of the above fractions showed an enhanced peak at t_R 2.5 min. The mobile phase (9:1 acetonitrile−water) for the HPLC analyses was free of Me_2CO (analytical grade solvents).

Treatment of Cryptotanshinone (3) with Acetone under Basic Conditions. To a stirred solution of **3**[8,9] (23 mg, 0.077 mmol) in EtOH (8 mL) were successively added Me_2CO (2 mL) and an EtOH solution of KOH (10%, w/v, 2 mL, 3.56 mmol), and the reaction mixture was stirred at room temperature for 8 min. Then, water (20 mL) was added and the mixture was extracted with CH_2Cl_2 (4 × 15 mL). The extracts were dried (Na_2SO_4) and filtered, and the solvents were removed, giving a residue (23 mg), which was chromatographed (Si gel 230−400 mesh column, 7 g, eluted with 4:1 petroleum ether−EtOAc), yielding pure **2** (17 mg, 0.048 mmol, 61.8%), less polar (TLC, 4:1 petroleum ether−EtOAc as eluent) than the starting material (**3**).

(12*R*,13*S*,14*S*,15*R*)-14,16-Epoxy-12α-hydroxy-12*β*,14*β*-(2-oxopropan-1,3-diyl)-20-nor-abieta-5(10),6,8-trien-11-one (2): rectangular white plates (EtOAc−*n*-pentane); mp 258−261 °C; $[\alpha]^{24}_D$ −44.5° (*c* 0.137, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 215 (4.43), 258 (4.07), 308 (3.51) nm; IR (KBr) ν_{max} 3424, 3101, 2959, 2929, 1721, 1677, 1590, 1267, 1228, 1126, 1045, 974, 942, 852 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.63 (1H, d, J = 8.4 Hz, H-6), 7.42 (1H, d, J = 8.4 Hz, H-7), 4.63 (1H, s, HO-12α), 3.90 (1H, t, $J_{gem} = J_{16\alpha,15\alpha}$ = 8.6 Hz, H-16α), 3.59 (1H, dd, J_{gem} = 8.6 Hz, $J_{16\beta,15\alpha}$ = 7.3 Hz, H-16β), 3.19 (1H, ddd, J_{gem} = 15.4 Hz, $J_{a,a'}$ = 9.8 Hz, $J_{a,e'}$ = 5.8 Hz, axial H-1a), 2.92 (1H, d, J_{gem} = 14.5 Hz, axial pro-*R* H-23a), 2.90 (1H, m,* equatorial H-1b),[32] 2.86 (1H, dd, J_{gem} = 14.5 Hz, $J_{23b,21b}$ = 1.5 Hz, equatorial pro-*S* H-23b), 2.77 (1H, d, J_{gem} = 17.0 Hz, axial pro-*S* H-21a), 2.62 (1H, d, $J_{13\beta,15\alpha}$ = 10.5 Hz, H-13β), 2.53 (1H, dd, J_{gem} = 17.0 Hz, $J_{21b,23b}$ = 1.5 Hz, equatorial pro-*R* H-21b), 2.05 (1H, dddq, $J_{15\alpha,13\beta}$ = 10.5 Hz, $J_{15\alpha,16\alpha}$ = 8.6 Hz, $J_{15\alpha,16\beta}$ = 7.3 Hz, $J_{15\alpha,17}$ = 6.8 Hz, H-15α), 1.83 (1H, m,* H-2a), 1.66 (1H, m,* H-2b), 1.60 (2H, m,* H_2-3), 1.28 (3H, s, Me-18 or Me-19), 1.22 (3H, s, Me-19 or Me-18), 1.21 (3H, d, $J_{17,15\alpha}$ = 6.8 Hz, Me-17); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.5 (C, C-22), 199.4 (C, C-11), 147.6 (C, C-5), 145.0 (C, C-8), 140.2 (C, C-10), 134.7 (CH, C-6), 124.9 (C, C-9), 124.2 (CH, C-7), 82.3 (C, C-14), 76.1 (C, C-12), 75.2 (CH_2, C-16), 61.6 (CH, C-13), 54.8 (CH_2, C-23), 52.6 (CH_2, C-21), 38.0 (CH_2, C-3), 34.4 (CH, C-15), 34.2 (C, C-4), 31.87 (CH_3, C-18 or C-19), 31.85 (CH_3, C-19 or C-18), 30.0 (CH_2, C-1), 19.3 (CH_2, C-2), 18.6 (CH_3, C-17); EIMS *m/z* 354 $[M]^+$ (100), 336 (63), 321 (13), 308 (16), 294 (39), 291 (93), 266 (29), 251 (31), 237 (30), 223 (21), 165 (18), 141 (13), 128 (15), 115 (11), 83 (8), 69 (6), 55 (7), 43 (7), 41 (5); *anal*. C 74.71%, H 7.26%, calcd for $C_{22}H_{26}O_4$, C 74.55%, H 7.39%.

Attempts at Obtaining Michael Adducts from 7α-Acetoxyroyleanone (5) and Acetone. Treatment of **5**,[23−25] as described above for obtaining **2**, yielded the starting material (**5**) and minute amounts (>4%) of horminone (**6**),[24,25] identified by comparison (TLC, UV, and MS) with an authentic sample.[5]

When a solution of **5** (39 mg, 0.026 mmol) in MeOH−Me_2CO (2:1, v/v, 30 mL) was treated with K-*t*-BuO (19 mg, 0.168 mmol) at room temperature for 48 h, the starting material (**5**) was recovered unchanged.

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References and Notes

(1) Connolly, J. D.; Hill, R. A. *Dictionary of Terpenoids*; Chapman & Hall: London, 1991; Vol. 2, pp 810−851.
(2) Abdel-Mogib, M.; Albar, H. A.; Batterjee, S. M. *Molecules* **2002**, *7*, 271−301.
(3) Hanson, J. R. *Nat. Prod. Rep.* **2004**, *21*, 785−793.
(4) Uchida, M.; Miyase, T.; Yoshizaki, F.; Bieri, J. H.; Rüedi, P.; Eugster, C. H. *Helv. Chim. Acta* **1981**, *64*, 2227−2250.
(5) Teixeira, A. P.; Batista, O.; Simões, M. F.; Nascimento, J.; Duarte, A.; De la Torre, M. C.; Rodríguez, B. *Phytochemistry* **1997**, *44*, 325−327.
(6) Gaspar-Marques, C.; Pedro, M.; Simões, M. F.; Nascimento, M. S. J.; Pinto, M. M. M.; Rodríguez, B. *Planta Med.* **2002**, *68*, 839−840.
(7) Cerqueira, F.; Cordeiro-Da-Silva, A.; Gaspar-Marques, C.; Simões, M. F.; Pinto, M. M. M.; Nascimento, M. S. J. *Bioorg. Med. Chem.* **2004**, *12*, 217−223.
(8) Tomita, Y.; Ikeshiro, Y. *J. Chem. Soc., Chem. Commun.* **1987**, 1311−1313.
(9) De la Torre, M. C.; Bruno, M.; Rodríguez, B.; Savona, G. *Phytochemistry* **1992**, *31*, 3953−3955.
(10) Hensch, M.; Rüedi, P.; Eugster, C. H. *Helv. Chim. Acta* **1975**, *58*, 1921−1934.
(11) Yoshizaki, F.; Rüedi, P.; Eugster, C. H. *Helv. Chim. Acta* **1979**, *62*, 2754−2762.
(12) Alder, A. C.; Rüedi, P.; Eugster, C. H. *Helv. Chim. Acta* **1984**, *67*, 1523−1530.
(13) Miyase, T.; Rüedi, P.; Eugster, C. H. *Helv. Chim. Acta* **1977**, *60*, 2770−2779.
(14) López, M. A.; Rodríguez, B.; Valverde, S. *An. Quím.* **1976**, *72*, 578−581.
(15) In this and previous studies[4−6] on *P. grandidentatus*, coleon U-quinone (**4**)[12] has not been reported among the diterpene constituents of this plant species.
(16) Luis, J. G.; Lahlou, E. H.; Andrés, L. S. *Tetrahedron* **1996**, *52*, 12309−12312.
(17) Luis, J. G.; Lahlou, E. H.; Andrés, L. S.; Sood, G. H. N.; Ripoll, M. M. *Tetrahedron Lett.* **1996**, *37*, 4213−4216.
(18) Tezuka, Y.; Kasimu, R.; Basnet, P.; Namba, T.; Kadota, S. *Chem. Pharm. Bull.* **1997**, *45*, 1306−1311.
(19) Nagy, G.; Günther, G.; Máthe, I.; Blunden, G.; Yang, M.-E.; Crabb, T. A. *Biochem. Syst. Ecol.* **1998**, *26*, 797−799.
(20) Nagy, G.; Günther, G.; Máthe, I.; Blunden, G.; Yang, M.-E.; Crabb, T. A. *Phytochemistry* **1999**, *52*, 1105−1109.
(21) Mei, S.-X.; Jiang, B.; Niu, X.-M.; Li, M.-L.; Yang, H.; Na, Z.; Lin, Z.-W.; Li, C.-M.; Sun, H.-D. *J. Nat. Prod.* **2002**, *65*, 633−637.
(22) Luo, D.; Zhang, X.; Tian, X.; Liu, J. *Helv. Chim. Acta* **2003**, *86*, 2784−2786.
(23) Hueso-Rodríguez, J. A.; Jimeno, M. L.; Rodríguez, B.; Savona, G.; Bruno, M. *Phytochemistry* **1983**, *22*, 2005−2009.
(24) Edwards, O. E.; Feniak, G.; Los, M. *Can. J. Chem.* **1962**, *40*, 1540−1546.
(25) Janot, M. M.; Potier, P. *Ann. Pharm. Fr.* **1964**, *22*, 387−395.
(26) Tezuka, Y.; Kasimu, R.; Li, J. X.; Basnet, P.; Tanaka, K.; Namba, T.; Kadota, S. *Chem. Pharm. Bull.* **1998**, *46*, 107−112.
(27) Scott, A. I. *Interpretation of the Ultraviolet Spectra of Natural Products*; Pergamon Press: London, 1964; pp 100−113.
(28) Rodríguez, B. *Magn. Reson. Chem.* **2003**, *41*, 741−746.
(29) Huang, W. G.; Jiang, Y. Y.; Li, Q.; Li, J.; Li, J. Y.; Lu, W.; Cai, J. C. *Tetrahedron* **2005**, *61*, 1863−1870.
(30) Bhacca, N. S.; Williams, D. H. *Applications of NMR Spectroscopy in Organic Chemistry*. Illustrations from the Steroid Field; Holden-Day: San Francisco, 1966; pp 115−123.
(31) Rodríguez, B.; Jimeno, M. L. *Magn. Reson. Chem.* **2004**, *42*, 605−616.
(32) Signals marked with an asterisk appeared as overlapped or partially overlapped multiplets, and their assignments were in agreement with the HSQC spectrum.

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