Labdane and Kaurane Diterpenoids from *Plectranthus fruticosus*

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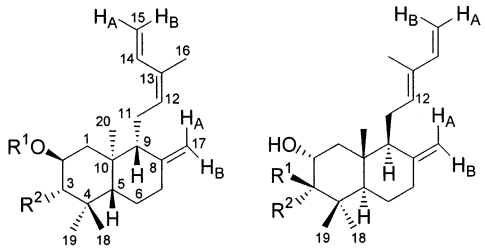
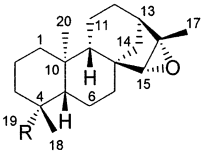
Six new diterpenoids, three labdane and three kaurane derivatives, have been isolated from an acetone extract of *Plectranthus fruticosus* together with other already known substances. The structures of these compounds (**1**−**5** and **7**) were established mainly by spectroscopic means, particularly by 1D and 2D NMR studies, as well as by some chemical correlations with known diterpenoids. The physical and spectroscopic data of the derivative **11**, obtained by hydrolysis of the labdane **2**, were identical to those reported for a compound previously isolated from *Croton joufra*, to which structure **12** had been established erroneously by other authors. Several of the isolated compounds were tested as antimicrobial agents against three bacteria strains and one yeast strain, but only **4** showed a moderate inhibitory activity against *Staphylococcus aureus*.

In continuation of our studies on biologically active diterpenoids from *Plectranthus* species (Labiatae),[1−4] we have now investigated *P. fruticosus* L'Hérit., a species that has not hitherto been studied in detail chemically or pharmacologically, with the exception of its leaf essential oil,[5] which has been shown to exhibit teratogenic effects.[6] In this paper, we report on the isolation and structure elucidation of six new diterpenoids, three labdanes (**1**−**3**) and three kaurane derivatives (**4**, **5**, and **7**), found in the acetone extract of the aerial parts of the plant. One of these kauranes (**5**) has been reported already as a synthetic compound,[7] and the deacetyl derivative **11**, obtained from the labdane **2** by alkaline hydrolysis, is identical to a substance recently isolated from *Croton joufra*, for which structure **12** was attributed.[8] In addition, we also report bioassay results on several of the new diterpenoids as antimicrobial agents.

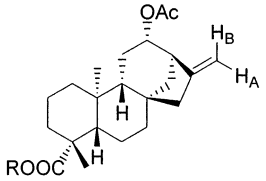


1 $R^1 = R^2 = H$
2 $R^1 = Ac$, $R^2 = OH$
10 $R^1 = Ac$, $R^2 = H$
11 $R^1 = H$, $R^2 = OH$

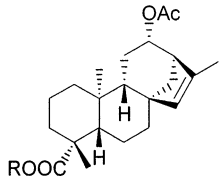
3 $R^1 = OAc$, $R^2 = H$
12 $R^1 = H$, $R^2 = OH$



4 R = COOH
5 R = CH_2OH
13 R = COOMe



6 R = H
8 R = Me



7 R = H
9 R = Me

Results and Discussion

Repeated chromatographic processes on the acetone extract of the aerial parts of *P. fruticosus* allowed the isolation of the diterpenoids **1**−**5** and a mixture of **6** and **7**, together with caryophyllene α-oxide,[9−12] a mixture of *β*-sitosterol and stigmasta-5,22*E*-dien-3*β*-ol,[13] and *β*-amyrin.[14,15] The mixture of **6** and **7**, after esterification with diazomethane, was easily separated into its constituents (methyl esters **8** and **9**, respectively) by column chromatography over Si gel impregnated with $AgNO_3$.

Combustion analysis and low-resolution mass spectrometry established a molecular formula $C_{20}H_{32}O$ for the first of the new diterpenoids (**1**), and its IR spectrum showed hydroxyl (3351 cm^{-1}), exocyclic methylene (3085, 892 cm^{-1}), and vinyl group (3085, 1643, 989 cm^{-1}) absorptions. The ^1H NMR spectrum of **1** displayed signals for a secondary hydroxyl group in an equatorial configuration in a cyclohexane ring and placed between two methylene groups (axial geminal proton at *δ* 3.88, 1H, tt, $J_{a,a'} = J_{a,a''} = 11.6$ Hz, $J_{a,e'} = J_{a,e''} = 4.0$ Hz), three methyl groups attached to fully substituted sp^3 carbons (*δ* 0.94, 0.85, and 0.76, 3H each, singlets), another methyl group on a trisubstituted olefinic double bond (*δ* 1.76, 3H, d, $J = 1.2$ Hz, olefinic proton at *δ* 5.30, 1H, br t, $J_{vic} = 6.6$ Hz), and an exocyclic methylene (*δ* 4.85 and 4.51, 1H each). In addition, the ^1H NMR spectrum of **1** showed signals for a vinyl group (*δ* 6.78, 1H, ddd, $J_{cis} = 10.8$ Hz, $J_{trans} = 17.2$ Hz, olefinic methine proton; *δ* 5.17, 1H, ddd, $J_{gem} = 1.6$ Hz, $J_{trans} = 17.2$ Hz, one of the olefinic methylene protons; *δ* 5.08, 1H, dt, $J_{gem} = 1.6$ Hz, $J_{cis} = 10.8$ Hz, the other olefinic methylene proton), whose protons displayed long-range coupling ($J_{allylic} = 0.8$ Hz, and $J_{homoallylic} = 0.6$ and 1.6 Hz for the olefinic methylene protons at *δ* 5.17 and 5.08, respectively)

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Table 1. ^{13}C NMR Spectral Data for Compounds **1**, **2**, **4**, **5**, **8**−**10**, and **13**[a]

carbon	**1**	**2**	**4**	**5**	**8**	**9**	**10**	**13**
C-1	48.37 (CH_2)	42.31 (CH_2)	40.77 (CH_2)	40.54 (CH_2)	40.57 (CH_2)	40.53 (CH_2)	44.27 (CH_2)	40.80 (CH_2)
C-2	65.64 (CH)	73.26 (CH)	18.96 (CH_2)	18.12 (CH_2)	18.91 (CH_2)	18.98 (CH_2)	69.27 (CH)	19.01 (CH_2)
C-3	51.11 (CH_2)	80.56 (CH)	37.79 (CH_2)	35.52 (CH_2)	37.93 (CH_2)	38.02 (CH_2)	46.82 (CH_2)	38.07 (CH_2)
C-4	35.06 (C)	39.88 (C)	43.73 (C)	38.62 (C)[b]	43.85 (C)[c]	43.90 (C)	34.96 (C)	43.80 (C)
C-5	54.78 (CH)	54.43 (CH)	56.73 (CH)	56.50 (CH)	56.80 (CH)	56.61 (CH)	54.87 (CH)	56.69 (CH)
C-6	23.79 (CH_2)	23.56 (CH_2)	20.56 (CH_2)	19.19 (CH_2)	21.77 (CH_2)	20.69 (CH_2)	23.71 (CH_2)	20.65 (CH_2)
C-7	37.86 (CH_2)	37.69 (CH_2)	35.71 (CH_2)	36.09 (CH_2)	41.02 (CH_2)	39.08 (CH_2)	37.79 (CH_2)	35.70 (CH_2)
C-8	147.66 (C)	146.89 (C)	43.73 (C)	43.64 (C)	43.18 (C)[c]	48.02 (C)	147.37 (C)	43.68 (C)
C-9	57.23 (CH)	56.88 (CH)	49.61 (CH)	50.63 (CH)	55.26 (CH)	48.17 (CH)	57.17 (CH)	49.53 (CH)
C-10	41.02 (C)	40.22 (C)	39.44 (C)	38.97 (C)[b]	38.42 (C)	38.24 (C)	40.88 (C)	39.19 (C)
C-11	22.25 (CH_2)	22.33 (CH_2)	18.26 (CH_2)	18.07 (CH_2)	23.25 (CH_2)	25.18 (CH_2)	22.26 (CH_2)	18.23 (CH_2)
C-12	131.18 (CH)	130.79 (CH)	26.96 (CH_2)	26.96 (CH_2)	73.79 (CH)	69.29 (CH)	131.12 (CH)	26.96 (CH_2)
C-13	131.81 (C)	131.97 (C)	39.08 (CH)	39.16 (CH)	48.01 (CH)	49.14 (CH)	131.85 (C)	39.02 (CH)
C-14	133.81 (CH)	133.77 (CH)	32.13 (CH_2)	32.02 (CH_2)	33.78 (CH_2)	37.50 (CH_2)	133.87 (CH)	32.10 (CH_2)
C-15	113.37 (CH_2)	113.48 (CH_2)	68.06 (CH)	68.29 (CH)	49.00 (CH_2)	138.32 (CH)	113.34 (CH_2)	68.04 (CH)
C-16	19.73 (CH_3)	19.65 (CH_3)	61.40 (C)	61.32 (C)	150.98 (C)	141.20 (C)	19.68 (CH_3)	61.37 (C)
C-17	108.48 (CH_2)	108.97 (CH_2)	14.56 (CH_3)	14.54 (CH_3)	106.34 (CH_2)	15.62 (CH_3)	108.74 (CH_2)	14.59 (CH_3)
C-18	33.69 (CH_3)	28.70 (CH_3)	28.92 (CH_3)	27.02 (CH_3)	28.83 (CH_3)	28.79 (CH_3)	33.60 (CH_3)	28.71 (CH_3)
C-19	22.69 (CH_3)	16.49 (CH_3)	183.69 (C)	65.37 (CH_2)	178.07 (C)	178.02 (C)	22.51 (CH_3)	177.98 (C)
C-20	15.36 (CH_3)	15.21 (CH_3)	15.29 (CH_3)	17.92 (CH_3)	13.80 (CH_3)	13.26 (CH_3)	15.19 (CH_3)	15.10 (CH_3)
$-OCOCH_3$		171.62 (C)			170.42 (C)	170.70 (C)	170.61 (C)	
$-OCOCH_3$		21.36 (CH_3)			21.60 (CH_3)	21.60 (CH_3)	21.50 (CH_3)	
$-COOCH_3$					51.20 (CH_3)	51.16 (CH_3)		51.16 (CH_3)

[a] In $CDCl_3$ solution, at 100 MHz. All these assignments were in agreement with HSQC and HMBC spectra. [b,c] These assignments are reversed with respect to those reported previously.[7,30]

with the olefinic proton resonating at δ 5.30. The UV absorption of **1** at 238 nm (log ϵ 4.08) supported the presence of the 1,3-diene chromophore in this diterpenoid. The ^{13}C NMR spectrum of **1** (Table 1) was very similar to that reported[16] for labda-8(17),12*Z*,14-triene, showing almost identical resonances for the C-5 through C-20 carbon atoms, whereas the observed differences in the chemical shifts of the C-1−C-4 carbons [$\Delta\delta = \delta$(**1**) − δ(ref 14): +10.2, +46.1, +11.8, and +1.4 ppm, respectively] were compatible with the presence in **1** of an equatorial hydroxyl substituent at the C-2 position.[17]

The ^1H and ^{13}C NMR data corresponding to the C-9 side chain of **1** established that this substance possesses a C-12, C-13 olefinic double bond in a *Z*-configuration, because the proton and carbon atom resonances of the C-11−C-16 structural part were almost identical to those reported for some structurally related diterpenoids with a 12*Z*-configuration and very different from data observed for the 12*E* isomers.[16−20] This conclusion was also in agreement with NOE experiments on **1**, because irradiation at δ 5.30 (H-12) caused a noticeable NOE enhancement (+6.2%) in the signal of the Me-16 group (δ 1.76) and not in the signal of the H-14 proton (δ 6.78). On the other hand, NOE experiments also supported the relative stereochemistry of the decalin part of **1**, as is depicted in the formula.[21] Irradiation at δ 3.88 (axial proton geminal to the hydroxyl substituent) produced NOE enhancements in the signals of the H-1β (δ 2.12, +2.4% NOE enhancement), H-3β (δ 1.75, +2.0%), Me-19 (δ 0.85, +2.2%), and Me-20 (δ 0.76, +3.2%) protons, but not in the signal of the H-5α proton (δ 1.10), thus establishing that all these hydrogens, except for H-5α, are on the same side of the plane defined by the decalin (*ent*-β-side in formula **1**[21]) and that the A/B decalin junction is *trans*. The absence of any NOE enhancement in the signal of the H-5α proton when the Me-20 protons were irradiated further supported this point. In addition, NOE experiments allowed the unambiguous assignment of both C-17 methylene protons, because irradiation at δ 4.85 (H_B-17) caused a NOE enhancement (+4.2%) in the signal of the H-7β proton (δ 2.39), thus establishing that H_B-17 was the pro-*E* hydrogen.

All of the above data, together with 2D NMR experiments (COSY, TOCSY, HSQC, and HMBC), established a structure such as **1** for this diterpenoid, except for its absolute configuration, which was not ascertained by direct methods. However, the change of the molecular rotation of **1** (M_D −116) with respect to that of its acetyl derivative (**10**, M_D −35) is opposite of those reported for 2α-hydroxy-5α-steroids and their corresponding acetates, in which the acetylation causes a negative increment in the M_D value.[22] This behavior seems to indicate that **1** possesses the *enantio* absolute stereochemistry depicted in its formula.

The second of the new diterpenoids isolated from *P. fruticosus* (**2**, $C_{22}H_{34}O_3$) showed ^1H and ^{13}C NMR spectra (see Experimental Section and Table 1, respectively) very similar to those of the acetyl derivative **10**. In fact, the C-5−C-17 and C-20 carbon atom resonances were identical in both compounds (Table 1), whereas the observed differences in the chemical shifts of the C-1−C-4, C-18, and C-19 carbons [$\Delta\delta = \delta$(**2**) − δ(**10**): −2.0, +4.0, +33.7, +4.9, −4.9, and −6.0 ppm, respectively] were in agreement with the presence in **2** of an additional hydroxyl group at the C-3 position. Moreover, the HMBC spectrum of **2** showed connectivities between the carboxyl carbon of the acetate (δ_C 171.62 s) and the H-2β proton (δ_H 4.94 ddd, δ_{C-2} 73.26 d), which in turn was connected with the C-1, C-3, C-4, and C-10 carbons (δ 42.31 t, 80.56 d, 39.88 s, and 40.22 s, respectively), and between the C-3 hydroxylic carbon and the H_2-1 (δ 1.24 dd and 2.11 dd), H-2β, H-5α (δ 1.20 dd), Me-18 (δ 1.05, 3H, s), and Me-19 (δ 0.85, 3H, s) protons. The 2α-acetoxy and 3β-hydroxy configurations[21] of **2** were in agreement with the coupling constant values observed for the H-1α, H-1β, H-2β, and H-3α (δ 3.22 d) protons ($J_{1\alpha,1\beta}$ = 12.3 Hz, $J_{1\alpha,2\beta}$ = 11.7 Hz, $J_{1\beta,2\beta}$ = 4.4 Hz, and $J_{2\beta,3\alpha}$ = 10.1 Hz). These coupling values are compatible only with a spatial arrangement in which the H-1α, H-2β, and H-3α protons are axial substituents and the H-1β proton is in an equatorial configuration.[23] NOE experiments further supported this conclusion, because irradiation at δ 3.22 (H-3α) caused strong NOE enhancements in the signals of the H-1α, H-5α, and Me-18 protons (+3.4, +3.6, and +3.6%, respectively) and a weak NOE (+0.9%) in the vicinal H-2β proton, whereas by irradiating at δ 4.94 (H-2β) the signals of the H-1α, H-1β, H-3α, Me-19, and Me-20 (δ 0.83) protons were affected (NOE enhancements +1.0, +3.2, +0.8, +1.6, and +1.9%, respectively). In addition, the 12*Z* stereochem-

istry of **2** was also in agreement with NOE experiments, because irradiation at δ 5.23 (H-12) produced a strong NOE enhancement (+6.1%) in the signal of the Me-16 protons (δ 1.76, 3H, d).

Alkaline hydrolysis of **2** yielded **11** ($C_{20}H_{32}O_2$), a substance whose melting point, IR, ^{1}H and ^{13}C NMR, and mass spectra were identical to those reported for a diterpenoid recently isolated from *Croton joufra* Roxb. (Euphorbiaceae), to which structure **12** was attributed, except for its absolute configuration.[8] Furthermore, the optical rotation of **11** ($[\alpha]^{20}_D$ −23.7°, *c* 0.313, $CHCl_3$) and that reported for **12** ($[\alpha]^{25}_D$ −18.24°, *c* 0.34, $CHCl_3$)[8] are very similar, thus suggesting that both compounds are identical. The ^{1}H and ^{13}C NMR data corresponding to the C-11−C-16 structural part of **11**, identical to those reported[8] for **12**, are compatible only with a 12*Z* stereochemistry[16−20] (such as in **1**, **2**, and **10**, see above) and not with the opposite one (12*E*) proposed for the substance (**12**) isolated from *C. joufra*.[8] Moreover, the ^{1}H and ^{13}C NMR data for the C-1−C-5 structural moiety of **11**[21] were also identical to those observed[8] for **12** and strongly supported a 2α,3β-dihydroxy configuration[23] for **11**, and therefore for the diterpenoid found in *C. joufra*. Furthermore, irradiation on the H-3α proton signal of **11** (δ 3.04) caused NOE enhancements in the signals of the H-1α (+2.3%), H-2β (+0.8%), H-5α (+4.1%), and Me-18 (+3.7%) protons, thus establishing an axial configuration for the H-1α, H-3α, and H-5α protons. From all these results, and particularly from the $J_{2\beta,3\alpha}$ *trans*-diaxial coupling value[23] (9.6 Hz for **11** and **12**[8]), it was evident that the C-3 hydroxyl substituent in **11**, and hence in the diterpene isolated from *C. joufra*,[8] possesses an equatorial configuration (*ent*-3β in the formula[21]), despite the weak NOE observed between the H-2β and H-3α protons (see above), a spectroscopic behavior that had been taken as the only argument[8] for supporting a 3α-hydroxy configuration in **12**. As in the case of **1**, the change in the molecular rotation of **11** (M_D −72) with respect to that of **2** (M_D +38) suggests an *enantio* absolute configuration for this new diterpenoid (ΔM_D due to acetylation +110).[22] From all the above data we also conclude definitely that structure **12**[8] must be amended to **11**.

Another of the new diterpenoids of *P. fruticosus* ($C_{22}H_{34}O_3$) possessed the structure and absolute stereochemistry depicted in **3**. Its ^{1}H and ^{13}C NMR spectra showed signals for a 3-methyl-1,3*E*-pentadien-5-yl structural moiety[16−20] and for a decalin part almost identical to that of **2**. The 3*E*-configuration of **3** was confirmed by NOE experiments because irradiation at δ 5.37 (1H, br t, H-12) produced, among others, a NOE enhancement (+7.0%) in the signal of the H-14 proton (δ 6.30, ddd) and not in the Me-16 signal (δ 1.73, 3H, d). The HMBC spectrum of **3** showed a cross-peak between the carbonyl carbon of the acetate (δ 172.42 s) and an axial proton resonating at δ 4.52 (d, H-3α), which was *trans*-diaxial coupled ($J_{3\alpha,2\beta}$ = 10.1 Hz) with the H-2β proton (δ 3.80 ddd, $J_{2\beta,1\alpha}$ = 11.6 Hz, $J_{2\beta,1\beta}$ = 4.4 Hz).

Application of Horeau's method[24] to **3** defined as *R* the configuration of the C-2 stereogenic center (see Experimental Section) and, consequently, a *normal* labdane absolute configuration for this diterpenoid. Recently, Roengsumran and co-workers[25] have isolated from *Croton oblongifolius* Roxb. a substance [mp 99−101 °C, $[\alpha]^{20}_D$ +9.46° (*c* 1.0, $CHCl_3$)] that showed IR, UV, ^{1}H and ^{13}C NMR, and mass spectra identical to those of **3** [thick oil, $[\alpha]^{20}_D$ −18.7° (*c* 0.573, $CHCl_3$)]. Taking into account the opposite sign of the $[\alpha]_D$ values of **3** and the constituent of *C. oblongifolius*, we suggest that the latter compound possesses an *enantio* absolute configuration, a structural feature not ascertained previously.[25] This was also supported on biogenetic grounds, because *ent*-labdanes have already been reported as constituents of other *Croton* species.[26]

ent-15β,16β-Epoxykauran-19-oic acid[21] (**4**, $C_{20}H_{30}O_3$) was also found in the acetone extract of *P. fruticosus*. Treatment of **4** with an ethereal solution of diazomethane yielded a substance (**13**, $C_{21}H_{32}O_3$) identical to a synthetic product described previously.[27] Moreover, reduction of **4** with $LiAlH_4$ gave the epoxyalcohol **5** ($C_{20}H_{32}O_2$), identical to another constituent of *P. fruticosus* and also known as a synthetic derivative obtained from *ent*-15-kauren-19-oic acid.[7] To the best of our knowledge, this is the first report on the isolation of **4** and **5** from a natural source. The complete and unambiguous assignments of the ^{1}H NMR spectra of **5** and **13**, not previously reported,[7,27] are included in the Experimental Section, together with other unpublished physical and spectroscopic data of these compounds. Moreover, the ^{13}C NMR spectrum of **13**, not reported before now,[27] and the correct assignments for the δ_C values of **5**[7] are shown in Table 1.

The acid **6**, characterized as its methyl ester derivative (**8**), was also found in *P. fruticosus*. These compounds are already known, in the case of **6** as a natural constituent of several plant species,[28−30] and **8** as a synthetic derivative.[28,30] The complete assignment of the ^{1}H NMR spectrum of **8** as well as the unambiguous reassignment of its ^{13}C NMR data[30] are included in the Experimental Section and Table 1, respectively.

The last of the new diterpenoids found in *P. fruticosus* (**7**) was isolated as its methyl ester derivative (**9**, $C_{23}H_{34}O_4$). The ^{1}H and ^{13}C NMR spectra of **9** were similar to those of **8**, showing characteristic signals for a kaur-15-ene structure [δ_H 5.11 (1H, qd, $J_{15,17}$ = 1.6 Hz, $J_{15,14\alpha}$ = 0.8 Hz, H-15) and 1.75 (3H, d, $J_{17,15}$ = 1.6 Hz, Me-17); δ_C 138.32 (d, C-15), 141.20 (s, C-16), and 15.62 (q, C-17)]. The observed differences in the chemical shifts of the C-7−C-9 and C-11−C-14 carbons in **8** and **9** (Table 1) further supported[31] this assumption. Finally, isomerization of the exocyclic olefin of **8** with I_2 as a catalyst[32,33] yielded a compound whose physical (mp, $[\alpha]_D$) and spectroscopic (^{1}H and ^{13}C NMR, and mass spectra) data were identical to those of **9**.

Caryophyllene α-oxide and **3**−**5**, **8**−**10**, and **13** were tested as antimicrobial agents against one Gram-positive and two Gram-negative bacteria and a yeast strain. None of the assayed compounds showed activity, except for **4**, which was moderately active against *Staphylococcus aureus* (MIC value 31.25 μg/mL). It was not possible to perform other assays of antimicrobial activity with the other compounds, due to the scarcity of the samples available.

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. IR spectra were obtained on a Perkin-Elmer Spectrum One spectrophotometer. UV spectra were recorded on a Perkin-Elmer Lambda 2 UV/vis 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, and chemical shifts are reported with respect to residual $CHCl_3$ (δ 7.25) for protons and to the solvent signals (δ_{CDCl3} 77.00) for carbons. 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 60 (70−230 mesh and 230−400 mesh) was used for column chromatography. Merck 5554 Kieselgel 60 F254 sheets were used for TLC analysis. Petroleum ether (bp 50−70 °C) was used for column chromatography.

Plant Material. *Plectranthus fruticosus* L'Hérit. (Labiatae) was cultivated in the Faculty of Pharmacy Hortum, Lisbon University, from seeds provided by the Herbarium of the Botanical Garden of Lisbon, Portugal. Aerial parts of this species were collected in June 1999, and voucher specimens were deposited in the Herbarium of the Botanical Center of the "Instituto de Investigação Científica Tropical", Lisbon (ref. C. Marques, S/N° LISC).

Extraction and Isolation. Dried and powdered *P. fruticosus* L'Hérit. aerial parts (3.58 kg) were extracted with Me_2CO (3 × 10 L) at room temperature for 8 days. After filtration and evaporation of the solvent under reduced pressure at low temperature (40 °C) a residue (444 g, 12.4% yield on dry plant material) remained. A part of this residue (100 g) was subjected to column chromatography (Si gel 70−230 mesh, 960 g) eluting successively with petroleum ether, petroleum ether−EtOAc (9:1, 3:1, 1:1), and EtOAc. The residue (5.5 g) of the fractions eluted with 9:1 petroleum ether−EtOAc was rechromatographed [Si gel 230−400 mesh column, 50 g, petroleum ether−EtOAc (49:1) as eluent], giving waxes and a crystalline substance (62 mg, 0.0077% on dry plant material). This compound was identified as (−)-caryophyllene oxide (caryophyllene α-oxide) by its physical (mp, $[\alpha]_D$) and spectroscopic (^{1}H and ^{13}C NMR, and mass spectra) data, which were identical to those reported previously for this sesquiterpenoid.[9−12]

The fractions from the initial chromatography eluted with 3:1 petroleum ether−EtOAc gave a residue (15 g). Rechromatography of this residue (Si gel 230−400 mesh column, 250 g, eluted with a solvent gradient from petroleum ether−EtOAc, 95:5 to 7:3) yielded, in order of increasing chromatographic polarity, the following compounds: **1** (11 mg, 0.0013% on dry plant material), a mixture of *β*-sitosterol and stigmasta-5,22*E*-dien-3*β*-ol[13] [287 mg, 0.035%; identified by the ^{1}H NMR spectrum of the mixture and by comparison (TLC) with authentic samples], **4** (162 mg, 0.02%), a mixture of **6** and **7** (1037 mg, 0.13%), **5** (15 mg, 0.0018%), **3** (45 mg, 0.0055%), impure **2** (65 mg), and *β*-amyrin (15 mg, 0.0018%; identified by its ^{1}H and ^{13}C NMR spectra).[14,15] The fraction containing impure **2** was rechromatographed [Si gel 70−230 mesh with 8% $AgNO_3$, 12 g; petroleum ether−EtOAc (4:1) as eluent], affording pure **2** (19 mg, 0.0023%, less polar compound on Si gel plus $AgNO_3$ plates) and additional quantities of **3** (10 mg, 0.0012%).

***ent*-Labda-8(17),12*Z*,14-trien-2α-ol (1):**[21] colorless thick oil; $[\alpha]^{22}_D$ −40.3° (*c* 0.149, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 238 (4.08) nm; IR (NaCl) ν_{max} 3351, 3085, 2931, 2852, 1660, 1643, 1461, 1439, 1388, 1368, 1034, 989, 892 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.78 (1H, ddd, $J_{14,12}$ = 0.8 Hz, $J_{14,15A}$ = 10.8 Hz, $J_{14,15B}$ = 17.2 Hz, H-14), 5.30 (1H, br t, $J_{12,11A}$ = $J_{12,11B}$ = 6.6 Hz, H-12), 5.17 (1H, ddd, $J_{15B,12}$ = 0.6 Hz, $J_{15B,14}$ = 17.2 Hz, $J_{15B,15A}$ = 1.6 Hz, H_B-15), 5.08 (1H, dt, $J_{15A,12}$ = $J_{15A,15B}$ = 1.6 Hz, $J_{15A,14}$ = 10.8 Hz, H_A-15), 4.85 (1H, q, $J_{17B,7\alpha}$ = $J_{17B,9\alpha}$ = $J_{17B,17A}$ = 1.6 Hz, H_B-17), 4.51 (1H, q, $J_{17A,7\alpha}$ = $J_{17A,9\alpha}$ = $J_{17A,17B}$ =1.6 Hz, H_A-17), 3.88 (1H, tt, $J_{2\beta,1\alpha}$ = $J_{2\beta,3\alpha}$ = 11.6 Hz, $J_{2\beta,1\beta}$ = $J_{2\beta,3\beta}$ = 4.0 Hz, H-2*β*), 2.44 (1H, ddd, $J_{11B,11A}$ = 17.5 Hz, $J_{11B,9\alpha}$ = 2.2 Hz, $J_{11B,12}$ = 6.6 Hz, H_B-11), 2.39 (1H, ddd, $J_{7\beta,7\alpha}$ = 13.2 Hz, $J_{7\beta,6\alpha}$ = 2.4 Hz, $J_{7\beta,6\beta}$ = 4.4 Hz, H-7*β*), 2.19 (1H, ddd, $J_{11A,11B}$ = 17.5 Hz, $J_{11A,9\alpha}$ = 10.2 Hz, $J_{11A,12}$ = 6.6 Hz, H_A-11), 2.12 (1H, ddd, $J_{1\beta,1\alpha}$ = 12.0 Hz, $J_{1\beta,2\beta}$ = 4.0 Hz, $J_{1\beta,3\beta}$ = 2.4 Hz, H-1*β*), 1.99 (1H, dddt, $J_{7\alpha,7\beta}$ = 13.2 Hz, $J_{7\alpha,6\alpha}$ = 5.3 Hz, $J_{7\alpha,6\beta}$ = 12.3 Hz, $J_{7\alpha,17A}$ = $J_{7\alpha,17B}$ = 1.6 Hz, H-7α), 1.76 (3H, d, $J_{16,12}$ = 1.2 Hz, Me-16), 1.76 (1H, ddt, $J_{9\alpha,11A}$ = 10.2 Hz, $J_{9\alpha,11B}$ = 2.2 Hz, $J_{9\alpha,17A}$ = $J_{9\alpha,17B}$ = 1.6 Hz, H-9α), 1.75 (1H, ddd, $J_{3\beta,3\alpha}$ = 12.0 Hz, $J_{3\beta,2\beta}$ = 4.0 Hz, $J_{3\beta,1\beta}$ = 2.4 Hz, H-3*β*), 1.72 (1H, dddd, $J_{6\alpha,6\beta}$ = 12.8 Hz, $J_{6\alpha,5\alpha}$ = 2.4 Hz, $J_{6\alpha,7\alpha}$ = 5.3 Hz, $J_{6\alpha,7\beta}$ = 2.4 Hz, H-6α), 1.30 (1H, dddd, $J_{6\beta,6\alpha}$ = 12.8 Hz, $J_{6\beta,5\alpha}$ = 12.4 Hz, $J_{6\beta,7\alpha}$ = 12.3 Hz, $J_{6\beta,7\beta}$ = 4.4 Hz, H-6*β*), 1.24 (1H, s, disappeared after addition of D_2O, OH-2α), 1.15 (1H, dd, $J_{3\alpha,3\beta}$ = 12.0 Hz, $J_{3\alpha,2\beta}$ = 11.6 Hz, H-3α), 1.10 (1H, dd, $J_{5\alpha,6\alpha}$ = 2.4 Hz, $J_{5\alpha,6\beta}$ = 12.4 Hz, H-5α), 1.04 (1H, dd, $J_{1\alpha,1\beta}$ = 12.0 Hz, $J_{1\alpha,2\beta}$ =11.6 Hz, H-1α), 0.94 (3H, s, Me-18), 0.85 (3H, s, Me-19), 0.76 (3H, s, Me-20); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m*/*z* 288 $[M]^+$ (18), 273 (10), 270 (35), 255 (98), 227 (38), 175 (55), 147 (48), 135 (69), 133 (70), 119 (77), 107 (80), 105 (80), 93 (87), 91 (89), 81 (89), 79 (100), 69 (49), 55 (59), 41 (67); *anal.* C 83.12%, H 11.09%, calcd for $C_{20}H_{32}O$, C 83.27%, H 11.18%.

***ent*-2α-Acetoxylabda-8(17),12*Z*,14-trien-3*β*-ol (2):**[21] colorless thick oil; $[\alpha]^{18}_D$ +10.9° (*c* 0.293, $CHCl_3$); IR (NaCl) ν_{max} 3486, 3086, 2944, 2871, 1732, 1644, 1439, 1370, 1252, 1055, 1030, 990, 958, 895, 757 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.75 (1H, ddd, $J_{14,12}$ = 0.8 Hz, $J_{14,15A}$ = 10.8 Hz, $J_{14,15B}$ = 17.3 Hz, H-14), 5.23 (1H, br t, $J_{12,11A}$ = $J_{12,11B}$ = 6.4 Hz, H-12), 5.17 (1H, ddd, $J_{15B,12}$ = 0.6 Hz, $J_{15B,14}$ = 17.3 Hz, $J_{15B,15A}$ = 1.6 Hz, H_B-15), 5.09 (1H, dt, $J_{15A,12}$ = $J_{15A,15B}$ = 1.6 Hz, $J_{15A,14}$ = 10.8 Hz, H_A-15), 4.94 (1H, ddd, $J_{2\beta,1\alpha}$ = 11.7 Hz, $J_{2\beta,1\beta}$ = 4.4 Hz, $J_{2\beta,3\alpha}$ = 10.1 Hz, H-2*β*), 4.87 (1H, q, $J_{17B,7\alpha}$ = $J_{17B,9\alpha}$ = $J_{17B,17A}$ =1.5 Hz, H_B-17), 4.50 (1H, q, $J_{17A,7\alpha}$ =$J_{17A,9\alpha}$ =$J_{17A,17B}$ = 1.5 Hz, H_A-17), 3.22 (1H, d, $J_{3\alpha,2\beta}$ = 10.1 Hz, H-3α), 2.40 (1H, ddd, $J_{7\beta,7\alpha}$ = 13.0 Hz, $J_{7\beta,6\alpha}$ = 2.4 Hz, $J_{7\beta,6\beta}$ = 4.2 Hz, H-7*β*), 2.32 (1H, ddd, $J_{11A,11B}$ = 17.6 Hz, $J_{11B,9\alpha}$ = 3.2 Hz, $J_{11B,12}$ = 6.4 Hz, H_B-11), 2.21 (1H, ddd, $J_{11A,11B}$ = 17.6 Hz, $J_{11A,9\alpha}$ = 11.2 Hz, $J_{11A,12}$ = 6.4 Hz, H_A-11), 2.11 (1H, dd, $J_{1\beta,1\alpha}$ = 12.3 Hz, $J_{1\beta,2\beta}$ = 4.4 Hz, H-1*β*), 2.09 (3H, s, OAc-2α), 2.00 (1H, br ddd, $J_{7\alpha,7\beta}$ = 13.0 Hz, $J_{7\alpha,6\alpha}$ = 5.2 Hz, $J_{7\alpha,6\beta}$ = 12.8 Hz, H-7α), 1.76 (3H, d, $J_{16,12}$ = 1.3 Hz, Me-16), 1.74 (1H, br dd, $J_{9\alpha,11A}$ = 11.2 Hz, $J_{9\alpha,11B}$ = 3.2 Hz, H-9α), 1.71 (1H, dddd, $J_{6\alpha,6\beta}$ = 12.8 Hz, $J_{6\alpha,5\alpha}$ = 2.8 Hz, $J_{6\alpha,7\alpha}$ = 5.2 Hz, $J_{6\alpha,7\beta}$ = 2.4 Hz, H-6α), 1.40 (1H, dddd, $J_{6\beta,6\alpha}$ = 12.8 Hz, $J_{6\beta,5\alpha}$ = 12.4 Hz, $J_{6\beta,7\alpha}$ = 12.8 Hz, $J_{6\beta,7\beta}$ = 4.2 Hz, H-6*β*), 1.24 (1H, dd, $J_{1\alpha,1\beta}$ = 12.3 Hz, $J_{1\alpha,2\beta}$ = 11.7 Hz, H-1α), 1.20 (1H, dd, $J_{5\alpha,6\alpha}$ = 2.8 Hz, $J_{5\alpha,6\beta}$ = 12.4 Hz, H-5α), 1.05 (3H, s, Me-18), 0.85 (3H, s, Me-19), 0.83 (3H, s, Me-20); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m*/*z* 346 $[M]^+$ (4), 331 (1), 328 (1), 286 (24), 271 (32), 253 (26), 203 (12), 187 (36), 173 (31), 159 (22), 147 (32), 135 (39), 133 (43), 121 (39), 119 (38), 109 (36), 107 (39), 105 (41), 93 (41), 91 (44), 81 (53), 79 (50), 69 (21), 55 (31), 43 (100), 41 (34); *anal.* C 76.38%, H 9.75%, calcd for $C_{22}H_{34}O_3$, C 76.26%, H 9.89%.

3*β*-Acetoxylabda-8(17),12*E*,14-trien-2α-ol (3): colorless thick oil; $[\alpha]^{20}_D$ −18.7° (*c* 0.573, $CHCl_3$). Compound **3** showed UV, IR, ^{1}H and ^{13}C NMR, and mass spectra identical to those reported[25] for its enantiomer: white solid, mp 99−101 °C; $[\alpha]^{20}_D$ +9.46° (*c* 1.0, $CHCl_3$); see text.

***ent*-15*β*,16*β*-Epoxykauran-19-oic acid (4):**[21] colorless needles (EtOAc−*n*-hexane), mp 199−201 °C; $[\alpha]^{22}_D$ −36.4° (*c* 0.421, $CHCl_3$); IR (KBr) ν_{max} 3430−2665 br, 2929, 2871, 1691, 1469, 1445, 1264, 1202, 984, 848, 840, 794 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.40 (1H, br, −COO*H*), 2.65 (1H, s, H-15α), 2.15 (1H, ddd, $J_{3\beta,3\alpha}$ = 14.0 Hz, $J_{3\beta,2\alpha}$ = 3.0 Hz, $J_{3\beta,2\beta}$ = 2.6 Hz, H-3*β*), 2.09 (1H, m*, H-13*β*), 1.86 (1H, m*, H-1*β*), 1.83 (1H, m*, H-2*β*), 1.79 (1H, m*, H-6α), 1.70 (1H, m*, H-7*β*), 1.63 (1H, m*, H-6*β*), 1.55 (1H, m*, H-12α), 1.50 (2H, m*, H-11α and H-11*β*), 1.48 (1H, m*, H-12*β*) 1.47 (1H, m*, H-14*β*), 1.41 (3H, s, Me-17), 1.39 (1H, m*, H-2α), 1.38 (1H, m*, H-7α), 1.23 (3H, s, Me-18), 1.10 (1H, m*, H-9α), 1.03 (1H, m*, H-14α), 1.02 (1H, m*, H-5α), 0.98 (1H, m*, H-3α), 0.92 (3H, s, Me-20), 0.84 (1H, td, $J_{1\alpha,1\beta}$ = $J_{1\alpha,2\beta}$ = 13.6 Hz, $J_{1\alpha,2\alpha}$ = 3.5 Hz, H-1α); signals marked with an asterisk appeared as overlapped multiplets and their assignments were supported by the HSQC spectrum; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m*/*z* 318 $[M]^+$ (91), 303 (55), 300 (26), 285 (24), 275 (48), 273 (57), 257 (86), 239 (28), 229 (32), 201 (23), 173 (26), 159 (37), 147 (38), 135 (79), 121 (86), 107 (100), 91 (83), 79 (71), 67 (41), 55 (40), 43 (54), 41 (35); *anal.* C 75.50%, H 9.68%, calcd for $C_{20}H_{30}O_3$, C 75.43%, H 9.50%.

***ent*-15*β*,16*β*-Epoxykauran-19-ol (5):**[21] colorless needles (EtOAc−*n*-hexane), mp 156−159 °C; $[\alpha]^{20}_D$ +2.3° (*c* 0.558, $CHCl_3$); IR (KBr) ν_{max} 3472, 2928, 2868, 2847, 1481, 1443, 1380, 1038, 986, 837, 796 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.71 (1H d, $J_{19B,19A}$ = 11.0 Hz, H_B-19), 3.41 (1H, dd, $J_{19A,19B}$ = 11.0 Hz, $J_{19A,3\alpha}$ = 1.2 Hz, H_A-19), 2.64 (1H, s, H-15α), 2.09 (1H, m*, H-13*β*), 1.84 (1H, dddd, $J_{1\beta,1\alpha}$ = 13.2 Hz, $J_{1\beta,2\alpha}$ = 3.6 Hz, $J_{1\beta,2\beta}$ = 3.2 Hz, $J_{1\beta,3\beta}$ = 1.6 Hz, H-1*β*), 1.78 (1H, dtd, $J_{3\beta,3\alpha}$ = 13.6 Hz, $J_{3\beta,2\alpha}$ = $J_{3\beta,2\beta}$ = 3.2 Hz, $J_{3\beta,1\beta}$ = 1.6 Hz, H-3*β*), 1.71 (1H, dt, $J_{7\beta,7\alpha}$ = 13.2 Hz, $J_{7\beta,6\alpha}$ = $J_{7\beta,6\beta}$ = 3.2 Hz, H-7*β*), 1.65 (1H, dtd,

$J_{6\alpha,6\beta}$ = 13.2 Hz, $J_{6\alpha,5\alpha}$ = 1.6 Hz, $J_{6\alpha,7\alpha} = J_{6\alpha,7\beta}$ = 3.2 Hz, H-6α), 1.57 (1H, m*, H-12α), 1.53 (3H, m*, H-11α, H-11*β*, and H-12*β*), 1.48 (1H, br d, $J_{14\beta,14\alpha}$ = 11.6 Hz, $J_{14\beta,13\beta}$ < 0.5 Hz, H-14*β*), 1.43 (1H, m*, H-7α), 1.41 (3H, s, Me-17), 1.40 (2H, m*, H-2α and H-2*β*), 1.20 (1H, dddd, $J_{6\beta,6\alpha}$ = 13.2 Hz, $J_{6\beta,5\alpha}$ = 12.4 Hz, $J_{6\beta,7\alpha}$ = 12.8 Hz, $J_{6\beta,7\beta}$ = 3.2 Hz, H-6*β*), 1.16 (1H, dd, $J_{9\alpha,11\alpha}$ = 4.0 Hz, $J_{9\alpha,11\beta}$ = 3.2 Hz, H-9α), 1.00 (1H, dt, $J_{14\alpha,14\beta}$ =11.6 Hz, $J_{14\alpha,13\beta} = J_{14\alpha,12\alpha}$ = 2.0 Hz, H-14α), 0.97 (3H, s, Me-20), 0.95 (3H, s, Me-18), 0.93 (1H, dd, $J_{5\alpha,6\alpha}$ = 1.6 Hz, $J_{5\alpha,6\beta}$ = 12.4 Hz, H-5α), 0.93 (1H, tdd, $J_{3\alpha,3\beta} = J_{3\alpha,2\beta}$ = 13.6 Hz, $J_{3\alpha,2\alpha}$ = 4.4 Hz, $J_{3\alpha,19A}$ = 1.2 Hz, H-3α), 0.81 (1H, td, $J_{1\alpha,1\beta} = J_{1\alpha,2\beta}$ = 13.2 Hz, $J_{1\alpha,2\alpha}$ = 3.6 Hz, H-1α); signals marked with an asterisk appeared as overlapped multiplets and their assignments were in agreement with the HSQC spectrum; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m*/*z* 304 $[M]^+$ (6), 286 (1), 273 (27), 255 (11), 191 (12), 177 (18), 159 (18), 149 (26), 135 (36), 123 (36), 107 (54), 105 (46), 91 (75), 81 (59), 79 (66), 67 (46), 55 (67), 43 (100), 41 (74); *anal*. C 78.79%, H 10.71%, calcd for $C_{20}H_{32}O_2$, C 78.89%, H 10.59%.

Compound **5** is already known as a synthetic derivative:[7] mp 149−151 °C, $[\alpha]_D$ not reported; partial ^{1}H NMR data and ^{13}C NMR spectrum identical to those obtained for **5**, except for the assignments of the C-4 and C-10 carbons (see Table 1 and ref 5).

Methylation of a Mixture of 6 and 7: Isolation of Methyl *ent*-12*β*-Acetoxy-16-kauren-19-oate (8) and Methyl *ent*-12*β*-Acetoxy-15-kauren-19-oate (9). A mixture of **6** and **7** isolated from the chromatographic process showed only one spot on TLC with several eluents. A part of this mixture (450 mg) was dissolved in Et_2O (50 mL) and then treated with an excess of an ethereal solution of CH_2N_2 at room temperature for 5 h. Evaporation of the solvent gave a mixture of the methyl esters **8** and **9** (455 mg, 99.4% yield), which were readily separated on a chromatographic column by using Si gel (70−230 mesh, 80 g) impregnated with 8% $AgNO_3$ (w/w) as adsorbent and petroleum ether−EtOAc (19:1) as eluent, yielding pure **8** (260 mg, less polar compound) and **9** (166 mg).

Methyl *ent*-12*β*-Acetoxy-16-kauren-19-oate (8):[21] colorless prisms (MeOH), mp 131−133 °C; $[\alpha]^{20}_D$ −37.8° (*c* 1.411, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 4.94 (1H, tt, $J_{17B,17A} = J_{17B,14\alpha}$ = 1.0 Hz, $J_{17B,15\alpha} = J_{17B,15\beta}$ = 2.0 Hz, H_B-17), 4.82 (1H, dq, $J_{17A,17B}$ = 1.0 Hz, $J_{17A,14\alpha} = J_{17A,15\alpha} = J_{17A,15\beta}$ = 0.8 Hz, H_A-17), 4.73 (1H, ddd, $J_{12\alpha,11\alpha}$ = 5.8 Hz, $J_{12\alpha,11\beta}$ = 1.2 Hz, $J_{12\alpha,13\beta}$ = 4.0 Hz, H-12α), 3.65 (3H, s, COOMe-19), 2.74 (1H, dd, $J_{13\beta,12\alpha}$ = 4.0 Hz, $J_{13\beta,14\alpha}$ = 4.6 Hz, $J_{13\beta,14\beta}$ = 0 Hz, H-13*β*), 2.22 (1H, d, $J_{14\beta,14\alpha}$ = 11.8 Hz, $J_{14\beta,13\beta}$ = 0 Hz, H-14*β*), 2.16 (1H, dddd, $J_{3\beta,3\alpha}$ = 13.5 Hz, $J_{3\beta,2\alpha}$ = 4.3 Hz, $J_{3\beta,2\beta}$ = 3.7 Hz, $J_{3\beta,1\beta}$ = 1.6 Hz, H-3*β*); 2.11 (2H, br dd, $J_{15,17A}$ = 0.8 Hz, $J_{15,17B}$ = 2.0 Hz, J_{gem} = 0 Hz, H_2-15), 2.02 (3H, s OAc-12*β*), 1.90 (1H, ddd, $J_{11\alpha,11\beta}$ = 16.9 Hz, $J_{11\alpha,9\alpha}$ = 9.6 Hz, $J_{11\alpha,12\alpha}$ = 5.8 Hz, H-11α), 1.81 (1H, m*, H-6α), 1.79 (1H, ddddd, $J_{2\beta,2\alpha}$ = 13.5 Hz, $J_{2\beta,1\alpha}$ = 13.1 Hz, $J_{2\beta,1\beta}$ = 3.8 Hz, $J_{2\beta,3\alpha}$ = 13.4 Hz, $J_{2\beta,3\beta}$ = 3.7 Hz, H-2*β*), 1.70 (2H, m*, H-1*β* and H-6*β*), 1.63 (1H, br dd, $J_{11\beta,11\alpha}$ = 16.9 Hz, $J_{11\beta,9\alpha}$ < 0.5 Hz, $J_{11\beta,12\alpha}$ = 1.2 Hz, H-11*β*), 1.59 (1H, dt, $J_{7\beta,7\alpha}$ =13.4 Hz, $J_{7\beta,6\alpha} = J_{7\beta,6\beta}$ = 3.2 Hz, H-7*β*), 1.47 (1H, td, $J_{7\alpha,7\beta} = J_{7\alpha,6\beta}$ = 13.4 Hz, $J_{7\alpha,6\alpha}$ = 4.1 Hz, H-7α), 1.39 (1H, ddddd, $J_{2\alpha,2\beta}$ = 13.5 Hz, $J_{2\alpha,1\alpha}$ = 4.0 Hz, $J_{2\alpha,1\beta}$ = 4.2 Hz, $J_{2\alpha,3\alpha}$ = 4.4 Hz, $J_{2\alpha,3\beta}$ = 4.3 Hz, H-2α), 1.19 (1H, br d, $J_{9\alpha,11\alpha}$ = 9.6 Hz, $J_{9\alpha,11\beta}$ < 0.5 Hz, H-9α), 1.17 (3H, s, Me-18), 1.04 (1H, ddt, $J_{14\alpha,14\beta}$ = 11.8 Hz, $J_{14\alpha,13\beta}$ = 4.6 Hz, $J_{14\alpha,17A} \cong J_{14\alpha,17B} \cong$ 0.9 Hz, H-14α), 1.03 (1H, dd, $J_{5\alpha,6\alpha}$ = 2.2 Hz, $J_{5\alpha,6\beta}$ = 11.6 Hz, H-5α), 0.97 (1H, ddd, $J_{3\alpha,3\beta}$ = 13.5 Hz, $J_{3\alpha,2\alpha}$ = 4.4 Hz, $J_{3\alpha,2\beta}$ = 13.4 Hz, H-3α), 0.88 (3H, s, Me-20), 0.76 (1H, td, $J_{1\alpha,1\beta} = J_{1\alpha,2\beta}$ = 13.1 Hz, $J_{1\alpha,2\alpha}$ = 4.0 Hz, H-1α); signals marked with an asterisk appeared as overlapped multiplets and their assignments were in agreement with the HSQC spectrum; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; IR and mass spectra identical to those reported previously;[28,30] ^{1}H NMR spectrum in agreement with the partial data reported in the literature;[28,30] ^{13}C NMR spectrum identical to that reported previously,[30] except for the assignments of the C-4 and C-8 carbons (see Table 1 and ref 30); lit. **8**[28,30] mp 126−128 °C, $[\alpha]_D$ −38° (*c* 1.9, $CHCl_3$).

Methyl *ent*-12*β*-Acetoxy-15-kauren-19-oate (9):[21] colorless needles (MeOH), mp 127−129 °C; $[\alpha]^{20}_D$ −12.7° (*c* 0.628, $CHCl_3$); IR (KBr) ν_{max} 3040, 2946, 2847, 1731, 1648, 1441, 1373, 1240, 1209, 1161, 1018, 964, 820 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 5.11 (1H, qd, $J_{15,14\alpha}$ = 0.8 Hz, $J_{15,17}$ = 1.6 Hz, H-15), 4.92 (1H, ddd, $J_{12\alpha,11\alpha}$ = 6.9 Hz, $J_{12\alpha,11\beta}$ = 1.0 Hz, $J_{12\alpha,13\beta}$ = 3.4 Hz, H-12α), 3.65 (3H, s, COOMe-19), 2.41 (1H, dd, $J_{13\beta,12\alpha}$ = 3.4 Hz, $J_{13\beta,14\alpha}$ = 4.0 Hz, $J_{13\beta,14\beta}$ = 0 Hz, H-13*β*), 2.30 (1H, d, $J_{14\beta,14\alpha}$ = 10.8 Hz, $J_{14\beta,13\beta}$ = 0 Hz, H-14*β*), 2.15 (1H, dddd, $J_{3\beta,3\alpha}$ = 13.5 Hz, $J_{3\beta,2\alpha}$ = 4.2 Hz, $J_{3\beta,2\beta}$ = 3.8 Hz, $J_{3\beta,1\beta}$ = 1.6 Hz, H-3*β*), 2.02 (3H, s, OAc-12*β*), 1.96 (1H, ddd, $J_{11\alpha,11\beta}$ = 16.5 Hz, $J_{11\alpha,9\alpha}$ = 9.7 Hz, $J_{11\alpha,12\alpha}$ = 6.9 Hz, H-11α), 1.81 (1H, ddt, $J_{2\beta,2\alpha}$ = 13.6 Hz, $J_{2\beta,1\alpha} = J_{2\beta3\alpha}$ = 13.1 Hz, $J_{2\beta,1\beta} = J_{2\beta,3\beta}$ = 3.8 Hz, H-2*β*), 1.80 (1H, dddd, $J_{6\alpha,6\beta}$ = 13.6 Hz, $J_{6\alpha,5\alpha}$ = 2.1 Hz, $J_{6\alpha,7\alpha}$ = 3.6 Hz, $J_{6\alpha,7\beta}$ = 3.2 Hz, H-6α), 1.75 (3H, d, $J_{17,15}$ = 1.6 Hz, Me-17), 1.70 (1H, dddd, $J_{6\beta,6\alpha}$ = 13.6 Hz, $J_{6\beta,5\alpha}$ = 11.7 Hz, $J_{6\beta,7\alpha}$ = 13.2 Hz, $J_{6\beta,7\beta}$ = 3.2 Hz, H-6*β*), 1.69 (1H, dddd, $J_{1\beta,1\alpha}$ = 13.1 Hz, $J_{1\beta,2\alpha}$ = 4.2 Hz, $J_{1\beta,2\beta}$ = 3.8 Hz, $J_{1\beta,3\beta}$ = 1.6 Hz, H-1*β*), 1.62 (1H, dt, $J_{7\beta,7\alpha}$ = 13.2 Hz, $J_{7\beta,6\alpha} = J_{7\beta,6\beta}$ = 3.2 Hz, H-7*β*), 1.52 (1H, td, $J_{7\alpha,7\beta} = J_{7\alpha,6\beta}$ = 13.2 Hz, $J_{7\alpha,6\alpha}$ = 3.6 Hz, H-7α), 1.51 (1H, br dd, $J_{11\beta,11\alpha}$ = 16.5 Hz, $J_{11\beta,9\alpha}$ < 0.5 Hz, $J_{11\beta,12\alpha}$ = 1.0 Hz, H-11*β*), 1.39 (1H, dqd, $J_{2\alpha,2\beta}$ = 13.6 Hz, $J_{2\alpha,1\alpha}$ = 4.1 Hz, $J_{2\alpha,1\beta} = J_{2\alpha,3\alpha} = J_{2\alpha,3\beta}$ = 4.2 Hz, H-2α), 1.23 (1H, ddd, $J_{14\alpha,14\beta}$ = 10.8 Hz, $J_{14\alpha,13\beta}$ = 4.0 Hz, $J_{14\alpha,15}$ = 0.8 Hz, H-14α), 1.16 (3H, s Me-18), 1.11 (1H, br d, $J_{9\alpha,11\alpha}$ = 9.7 Hz, $J_{9\alpha,11\beta}$ < 0.5 Hz, H-9α), 1.03 (1H, dd, $J_{5\alpha,6\alpha}$ = 2.1 Hz, $J_{5\alpha,6\beta}$ = 11.7 Hz, H-5α), 0.98 (1H, ddd, $J_{3\alpha,3\beta}$ = 13.5, $J_{3\alpha,2\alpha}$ = 4.2 Hz, $J_{3\alpha,2\beta}$ = 13.1 Hz, H-3α), 0.87 (3H, s, Me-20), 0.76 (1H, td, $J_{1\alpha,1\beta} = J_{1\alpha,2\beta}$ = 13.1 Hz, $J_{1\alpha,2\alpha}$ = 4.1 Hz, H-1α); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m*/*z* 374 $[M]^+$ (8), 359 (1), 314 (100), 299 (44), 282 (8), 255 (33), 239 (21), 207 (73), 185 (15), 159 (17), 146 (37), 131 (29), 121 (36), 105 (44), 92 (44), 91 (41), 81 (20), 67 (10), 55 (11), 43 (31), 41 (8); *anal*. C 73.83%, H 9.31%, calcd for $C_{23}H_{34}O_4$, C 73.76%, H 9.15%.

Preparation of *ent*-2α-Acetoxylabda-8(17),12*Z*,14-triene (10)[21] **from Compound 1.** Treatment of **1** (7 mg, 0.024 mmol) with Ac_2O−pyridine (1:1, 5 mL) at room temperature for 48 h yielded **10** (7 mg, 0.021 mmol, 87.5% yield) as a colorless thick oil: $[\alpha]^{20}_D$ −10.6° (*c* 0.541, $CHCl_3$); IR (NaCl) ν_{max} 3086, 2941, 2855, 1737, 1644, 1597, 1462, 1439, 1362, 1245, 1026, 989, 957, 893 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 6.77 (1H, ddd, $J_{14,12}$ = 0.8 Hz, $J_{14,15A}$ = 10.8 Hz, $J_{14,15B}$ = 17.4 Hz, H-14), 5.26 (1H, br t, $J_{12,11A} = J_{12,11B}$ = 6.3 Hz, H-12), 5.17 (1H, dd, $J_{15B,15A}$ = 1.6 Hz, $J_{15B,14}$ = 17.4 Hz, H_B-15), 5.09 (1H, dt, $J_{15A,15B} = J_{15A,12}$ = 1.6 Hz, $J_{15A,14}$ = 10.8 Hz, H_A-15), 5.02 (1H, tt, $J_{2\beta,1\alpha} = J_{2\beta,3\alpha}$ = 11.8 Hz, $J_{2\beta,1\beta} = J_{2\beta,3\beta}$ = 4.2 Hz, H-2*β*), 4.86 (1H, q, $J_{17B,17A} = J_{17B,7\alpha} = J_{17B,9\alpha}$ = 1.6 Hz, H_B-17), 4.49 (1H, q, $J_{17A,17B} = J_{17A,7\alpha} = J_{17A,9\alpha}$ = 1.6 Hz, H_A-17), 2.39 (1H, ddd, $J_{7\beta,7\alpha}$ = 13.0 Hz, $J_{7\beta,6\alpha}$ = 2.3 Hz, $J_{7\beta,6\beta}$ = 4.2 Hz, H-7*β*), 2.36 (1H, m*, H_B-11), 2.20 (1H, ddd, $J_{11A,11B}$ = 17.5 Hz, $J_{11A,9\alpha}$ = 11.0 Hz, $J_{11A,12}$ = 6.3 Hz, H_A-11), 2.09 (1H, ddd, $J_{1\beta,1\alpha}$ = 12.0 Hz, $J_{1\beta,2\beta}$ = 4.2 Hz, $J_{1\beta,3\beta}$ = 2.2 Hz, H-1*β*), 2.02 (3H, s, OAc-2α), 1.99 (1H, br ddd, $J_{7\alpha,7\beta}$ = 13.0 Hz, $J_{7\alpha,6\alpha}$ = 4.9 Hz, $J_{7\alpha,6\beta}$ = 12.8 Hz, H-7α), 1.77 (1H, m*, H-9α), 1.76 (3H, d, $J_{16,12}$ = 1.2 Hz, Me-16), 1.75 (1H, m*, H-3*β*), 1.71 (1H, m*, H-6α), 1.32 (1H, tdd, $J_{6\beta,6\alpha} = J_{6\beta,7\alpha}$ = 12.8 Hz, $J_{6\beta,5\alpha}$ = 12.5 Hz, $J_{6\beta,7\beta}$ = 4.2 Hz, H-6*β*), 1.25 (1H, dd, $J_{3\alpha,3\beta}$ = 12.1 Hz, $J_{3\alpha,2\beta}$ = 11.8 Hz, H-3α), 1.13 (1H, dd, $J_{5\alpha,6\alpha}$ = 2.6 Hz, $J_{5\alpha,6\beta}$ = 12.5 Hz, H-5α), 1.13 (1H, dd, $J_{1\alpha,1\beta}$ = 12.0 Hz, $J_{1\alpha,2\beta}$ = 11.8 Hz, H-1α), 0.94 (3H, s, Me-18), 0.90 (3H, s, Me-19), 0.81 (3H, s, Me-20); signals marked with an asterisk appeared as overlapped multiplets and their assignments were in agreement with the HSQC spectrum; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m*/*z* 330 $[M]^+$ (2), 270 (32), 255 (64), 227 (16), 214 (17), 199 (23), 187 (42), 175 (80), 159 (23), 147 (33), 135 (57), 133 (51), 119 (71), 105 (70), 93 (66), 91 (70), 81 (69), 79 (77), 67 (26), 55 (42), 43 (100), 41 (51); $C_{22}H_{34}O_2$ M_r 330.

Application of Horeau's Method[24] **to Compound 3.** Compound **3** (35.11 mg, 0.101 mmol) was treated with (±)-α-phenylbutyric anhydride (79.68 mg, 0.257 mmol) in pyridine solution (2.00 mL) for 18 h at room temperature: α_1 = +0.474, α_2 = +0.333, $\alpha_1 - 1.1\alpha_2$ = +0.108; configuration 2*R*.

Preparation of *ent*-Labda-8(17),12*Z*,14-triene-2α,3*β*-diol (11)[21] **from Compound 2.** A stirred solution of **2** (15 mg, 0.043 mmol) in EtOH (1 mL) was treated with an ethanolic solution of KOH (8%, w/v, 3 mL, 4.28 mmol) at room temperature for 12 h. Then, water (16 mL) was added to the reaction and the mixture was extracted with CH_2Cl_2 (10 mL × 5). The

extracts were dried (Na_2SO_4) and filtered and the solvents removed in vacuo, yielding a residue (11 mg, 0.036 mmol, 83.7%) of pure **11**: amorphous white solid, mp 76−80 °C; $[\alpha]^{20}_D$ −23.7° (*c* 0.313, $CHCl_3$); IR (KBr) ν_{max} 3413, 3086, 2968, 2854, 1644, 1439, 1386, 1097, 1054, 995, 953, 894 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 400 and 100 MHz, respectively) and mass spectra identical to those reported[8] for **12**: pale yellow powder, mp 72−74 °C; $[\alpha]^{25}_D$ −18.24° (*c* 0.34, $CHCl_3$).

Methylation of Compound 4 to Give Methyl *ent*-15*β*,16*β*-Epoxykauran-19-oate (13).[21] A solution of **4** (4 mg, 0.012 mmol) in Et_2O (30 mL) was treated with an excess of an ethereal solution of CH_2N_2 at room temperature for 3 h. After evaporation of the solvent, a residue (4 mg) remained. Crystallization from EtOAc−*n*-hexane yielded **13** (3.2 mg, 0.0096 mmol, 80%): colorless needles, mp 127−129 °C; $[\alpha]^{18}_D$ −36.5° (*c* 0.148, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.62 (3H, s, COOMe-19), 2.64 (1H, s, H-15α), 2.16 (1H, dddd, $J_{3\beta,3\alpha}$ = 13.2 Hz, $J_{3\beta,2\alpha}$ = 3.6 Hz, $J_{3\beta,2\beta}$ = 4.0 Hz, $J_{3\beta,1\beta}$ = 1.6 Hz, H-3*β*), 2.09 (1H, m, $W_{1/2}$ = 8 Hz, H-13*β*), 1.86 (1H, m*, H-1*β*), 1.83 (1H, m*, H-6α), 1.83 (1H, qt, $J_{2\beta,2\alpha} = J_{2\beta,1\alpha} = J_{2\beta,3\alpha}$ = 13.6 Hz, $J_{2\beta,1\beta} = J_{2\beta,3\beta}$ = 4.0 Hz, H-2*β*), 1.74 (1H, dt, $J_{7\beta,7\alpha}$ = 12.8 Hz, $J_{7\beta,6\alpha} = J_{7\beta,6\beta}$ = 2.9 Hz, H-7*β*), 1.55 (2H, m*, H-6*β* and H-12α), 1.52 (2H, m*, H-11α and H-11*β*), 1.49 (1H, br d, $J_{14\beta,14\alpha}$ = 11.2 Hz, $J_{14\beta,13\beta}$ < 0.5 Hz, H-14*β*), 1.45 (1H, m*, H-12*β*), 1.42 (1H, m*, H-2α), 1.41 (3H, s, Me-17), 1.40 (1H, m*, H-7α), 1.16 (3H, s, Me-18), 1.13 (1H, dd, $J_{9\alpha,11\alpha}$ = 4.3 Hz, $J_{9\alpha,11\beta}$ = 3.1 Hz, H-9α), 1.05 (1H, dd, $J_{14\alpha,14\beta}$ = 11.2 Hz, $J_{14\alpha,13\beta}$ = 2.0 Hz, H-14α), 1.02 (1H, dd, $J_{5\alpha,6\alpha}$ = 2.6 Hz, $J_{5\alpha,6\beta}$ = 12.0 Hz, H-5α), 1.00 (1H, ddd, $J_{3\alpha,3\beta}$ = 13.2 Hz, $J_{3\alpha,2\alpha}$ = 4.4 Hz, $J_{3\alpha,2\beta}$ = 13.6 Hz, H-3α), 0.83 (1H, td, $J_{1\alpha,1\beta} = J_{1\alpha,2\beta}$ = 13.6 Hz, $J_{1\alpha,2\alpha}$ = 3.6 Hz, H-1α), 0.80 (3H, s, Me-20); signals marked with asterisks appeared as overlapped multiplets and their assignments were in agreement with the HSQC spectrum; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m*/*z* 332 $[M]^+$ (19), 317 (12), 289 (23), 273 (39), 257 (31), 239 (16), 229 (16), 173 (18), 159 (26), 147 (27), 135 (58), 121 (100), 107 (79), 91 (90), 79 (81), 67 (53), 55 (69), 43 (93), 41 (73); $C_{21}H_{32}O_3$, M_r 332.

Compound **13** has previously been described as a synthetic derivative:[27] mp 123−127 °C; partial ^1H NMR data[27] identical to those reported above.

Reduction of Compound 4 to Afford Compound 5. To a solution of **4** (100 mg, 0.314 mmol) in anhydrous THF (15 mL) was added an excess of $LiAlH_4$ (200 mg, 5.27 mmol), and the reaction mixture was refluxed for 8 h under Ar. Workup in the usual manner yielded **5** (63 mg, 0.207 mmol, 65.9%, after crystallization from EtOAc−*n*-hexane), identical in all respects (mp, $[\alpha]_D$, ^1H NMR and mass spectra, TLC) with the compound isolated from the plant extract.

Transformation of Compound 8 into Compound 9. A solution of **8** (80 mg, 0.214 mmol) and I_2 (40 mg, 0.158 mmol) in benzene (40 mL) was refluxed for 24 h.[32,33] After washing the solution with 1% $Na_2S_2O_3$ and water, the benzene layer was dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue (76 mg), which was subjected to column chromatography [Si gel 70−230 mesh plus 8% $AgNO_3$, 28 g; petroleum ether−EtOAc (9:1) as eluent], yielding starting material (**8**, 32 mg, 0.088 mmol, 41%, less polar compound) and 41 mg (51%) of a substance which showed physical (mp, $[\alpha]_D$) and spectroscopic (^1H NMR and mass spectra) data identical to those of **9**.

Biological Assays. Antimicrobial activities of caryophyllene α-oxide, **3**−**5**, **8**−**10**, and **13** were tested against *Pseudomonas aeruginosa* ATCC 27853, *Escherischia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Candida albicans* CIP 3153A, obtained from the Microbiology Laboratory, Faculty of Pharmacy, Lisbon. The minimum inhibitory concentration (MIC) was performed by a broth microdilution method according to the NCCLS.[34] The tested compounds were dissolved in DMSO and graded concentration with a Mueller-Hinton broth ranging from 125 to 3.9 μg/mL.

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(12) An exhaustive NMR spectroscopic study on caryophyllene α-oxide, and particularly 1D NOESY experiments, allowed us to distinguish both methyl groups at the C-11 position, a spectroscopic assignment not previously reported.[10,11] The 11*β*-methyl group [C-12, *cis* with respect to the hydrogen of the epoxide (H-6)] resonates at δ_H 0.97 and δ_C 29.88, whereas the 11α-methyl group (C-13, *trans* with respect to H-6) appears at δ_H 1.00 and δ_C 21.62.
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(17) It is of interest to indicate that in labda-8(19),12*Z*,14-triene the assignments for the C-12 and C-14 carbons are reversed[16] with respect to those of **1** (Table 1). Our results are unambiguous, because they are based on the HSQC and HMBC spectra, and support a correct reassignment of the ^{13}C NMR data previously reported for several structurally related diterpenoids (see, for example, Garbarino, J. A.; Molinari, A. *J. Nat. Prod.* **1992**, *55*, 744−747; Furlan, M.; Lopes, M. N.; Fernandes, J. B.; Pirani, J. R. *Phytochemistry* **1996**, *41*, 1159−1161).
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(21) Since compounds **1**, **2**, **4**−**11**, and **13** belong to the *enantio* series (which is indicated by the prefix *ent*- before the complete name of the substance), the *ent*-α- or *ent*-*β*-configuration for a substituent indicates that it is placed, respectively, above or below the plane of the formulas depicted for these compounds. Moreover, the α- or *β*-configurations indicated for the assignment of the ^1H NMR spectra of these compounds also refer to this nomenclature throughout the text.
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