Acylphloroglucinol Derivatives from *Mahurea palustris*

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Five new acylphloroglucinol derivatives, mahureones A−E (**1**, **3**−**6**), have been isolated from the leaves of *Mahurea palustris*, and their structures determined by spectroscopic means. During the isolation process, several byproducts (**7**−**9**) were formed by reaction of one of the isoprenyl side chains with TFA, water, and acetonitrile. All the compounds were assayed for their ability to inhibit human DNA polymerase β. The most active compounds, mahureones A (**1**) and D (**5**), exhibited IC_{50} values in the 10 μM range.

DNA polymerase β (Pol β) is an essential enzyme involved in the DNA synthesis step of the base excision repair[1] and single-stranded break repair[2] processes. Because of the lack of associated proofreading activity, it is distinguishable from replicative DNA polymerases (Pol δ and Pol ϵ) by high infidelity in replicating DNA. It belongs to a newly identified family of mutagenic DNA polymerases whose cellular functions allow cells to adapt to genotoxic stresses by promoting DNA repair or to bypass DNA adducts or to participate in the immune response by promoting somatic hypermutation.[3] High levels of Pol β have been found in prostate, breast, and colon cancer tissues, and the possible involvement of Pol β in some tumorigenesis processes[4] and resistance to bifunctional alkylating agents[5] has been suggested because of its ability both to perturb the accurate replicative machinery[6] and to replicate across derived DNA adducts.[7] Inhibitors of Pol β have been sought actively among natural products[8] in the hope of finding use as adjunct therapy with, for example, *cis*-platinum or camptothecin derivatives. During the course of a large-scale screening of extracts to find new inhibitors of Pol β, we discovered an active nonpolar extract from the leaves of *Mahurea palustris*. Bioassay-guided fractionation of this extract led to a series of new acylphloroglucinol derivatives, and we report herein on their structure elucidation and on some unexpected reactions of these compounds during the purification stages.

Mahurea palustris Aublet is a tree growing in the coastal regions of the Guyanas and adjacent Brazil, belonging to the family Guttiferae (Clusiaceae) and the subfamily Kielmeyeroideae. It is one of two species of the genus *Mahurea*, which shares some common characteristics with the genus *Caraipa*, another endemic genus from Guyana and northern Brazil. Some taxonomists have included these two genera in the Theaceae or in the Bonnetiaceae family.[9] *M. palustris* was once used by the Palikur tribe as a war poison, and owing to the taste of its sap, it is commonly known as wine wood.[10]

Results and Discussion

Bioassay-guided fractionation led to the isolation of **1**, for which we propose the trivial name mahureone A (Figure 1). Its mass spectra showed a pseudomolecular ion at *m/z* 585 (positive mode, $[M + Na]^+$) or 561 (negative mode, $[M - H]^-$) for a $C_{35}H_{46}O_6$ composition. In the positive mode, the mass spectrum showed two prominent fragments corresponding to the successive losses of C_5H_9 (*m/z* 516) and $C_2H_3O_2$ (*m/z* 457). The UV spectrum showed the absorption of a phenyl ring (λ_{max} 283 nm), while the IR spectrum displayed vibrations for a complex polycarbonyl system. Examination of the literature suggested that this compound was similar to laxifloranone (**2**), also isolated from a member of the Guttiferae family, *Marila* laxiflora.[11]

Direct comparison of compounds **1** and **2** on TLC proved that they were different, but comparison of their ^1H and ^{13}C NMR spectra (Table 1) showed that these isomeric molecules had in common at least four elements: an isoprenyl chain, an isolavandulyl moiety, 3-methylbutyryl, and dihydrocinnamic acid moieties. Identification of these substituents was performed by analysis of 2D NMR experiments including COSY, HSQC, and HMBC (Figure 2). Thus, starting from the two olefinic proton triplets at δ 5.05 (H-16) and 5.18 (H-21), it was possible to assign the signals of two dimethylallyl chains, the former linked to a quaternary carbon at δ 46.7 (C-4) and the latter to a methine (C-6) at δ 41.2 (δ_H 1.63). The analysis was pursued from this signal on and demonstrated the presence of a rearranged lavanduloyl terpene unit linked at one end to the quaternary carbon C-4 bearing the first isoprenyl chain and at the other to a quaternary sp^2 center at δ 134.8 (C-8). The 3-methylbutyryl chain was characterized by a series of ^1H and ^{13}C NMR signals and was terminated by the carbonyl carbon at δ 201.3 (C-10). The dihydrocinnamic acid also formed a closed spin system, which could not be linked to the rest of the molecule through scalar couplings.

These elements accounted for a $C_{31}H_{45}O_3$ composition, thus leaving C_4HO_3 for the central core of the molecule. Of these four carbon atoms, three could be directly or indirectly detected (HMBC) and had shifts at δ 173.0,

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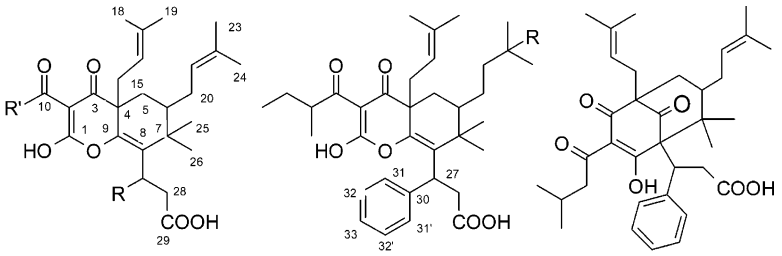


1 R = Ph R' = $(CH_3)_2CHCH_2$-
3 R = Ph R' = $CH_3CH_2CH(CH_3)$-
4 R = Ph R' = $(CH_3)_2CH$-
5 R = $CH_3CH_2CH_2$- R' = $(CH_3)_2CHCH_2$-
6 R = $CH_3CH_2CH_2$- R' = $CH_3CH_2CH(CH_3)$-

7 R = $NHCOCH_3$
8 R = OH
9 R = $OCOCF_3$

2 Laxifloranone

Figure 1. Structures of compounds **1**−**9**.

Table 1. ^{13}C NMR Data of Compounds **1**−**9**

carbon	**1**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	carbon	**1**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	173.0	160.4	161.0	nd	161.5	160.9	160.8	161.0	19	16.5	16.4	16.5	16.5	16.5	16.7	16.7	17.0
2	nd	100.6	100.0	nd	100.7	100.8	100.8	100.6	20	28.2	28.2	28.2	28.3	28.3	24.1	24.1	23.5
3	199.0	199. 1	199.3	nd	199.7	nd	198.0	nd	21	123.2	123.2	123.1	123.2	123.2	38.0	42.2	38.8
4	46.7	47.4	46.7	nd	46.8	46.7	46.7	46.6	22	132.6	132.6	132.6	132.5	132.5	53.2	69.9	89.4
5	28.6	28.6	28.7	28.8	28.8	29.3	29.3	29.3	23	24.8	24.7	24.7	16.5	16.5	26.1	27.6	24.5
6	41.2	41.2	41.2	41.0	41.2	40.9	40.9	40.8	24	16.5	16.6	16.6	24.7	24.7	26.1	27.6	24.2
7	38.9	38.9	38.9	38.7	38.6	39.2	39.2	39.1	25	24.9	24.8	24.7	24.3	24.3	24.7	24.7	24.5
8	134.8	134.4	134.6	133.0	132.9	134.3	134.3	134.0	26	19.8	19.8	19.7	19.2	19.2	19.6	19.4	19.4
9	143.5	143.7	143.7	nd	143.1	143.6	143.5	143.4	27	38.1	38.2	38.1	35.1	35.1	38.2	38.3	38.3
10	201.3	205.3	205.9	nd	206.5	205.7	205.7	206.0	28	36.9	36.9	36.9	38.8	38.8	36.9	36.9	37.1
11	45.5	40.8	33.9	45.7	41.1	40.9	40.9	40.8	29	174.9	174.9	174.9	175.4	175.3	174.9	174.9	nd
12	26.0	26.2	17.8	26.6	26.6	26.1	26.2	26.2	30	143.5	143.5	143.5	36.9	36.9	143.5	143.5	143.4
13	21.2[a]	10.8	17.6	21.4	10.7	11.3	11.0	11.0	31, 31′	126.3	127.6	126.4	21.0	21.0	126.4	126.5	126.4
14	21.7[b]	15.2	/	21.4	15.6	15.4	15.3	15.3	32, 32′	127.5	126.4	127.6	13.4	13.5	127.6	127.6	127.6
15	35.9	36.0	35.8	36.2	36.2	36.3	36.2	36.1	33	125.0	125.0	125.1		/	125.1	125.1	125.1
16	117.8	117.8	117.8	117.9	117.9	117.9	117.9	117.9	CO						171.2	/	155.0
17	136.1	135.7	135.7	135.6	135.6	135.9	135.9	135.9	Me/CF_3						22.0	/	nd
18	24.8	24.7	24.7	24.7	24.7	24.7	24.7	24.7									

[a,b] These resonances can be exchanged within the same column; nd: not determined.

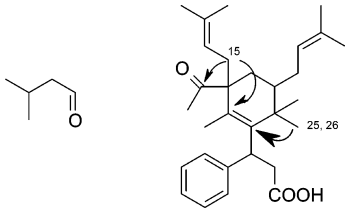


Figure 2. Elements of structure for mahureone A (**1**). Arrows indicate HMBC correlations.

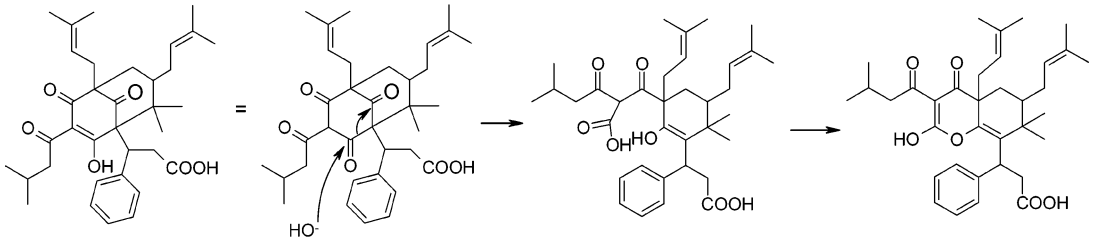
199.0, and 143.5 (overlapping with a phenyl ring atom). The signal at δ 143.5 belonged to the network of the isoprenyl chain and showed a ^3J coupling with the fortuitous equivalent methylene protons at δ 2.43; it was proposed to link it to the quaternary sp^2 center at δ 134.8 to form a six-membered ring. The carbonyl carbon at δ 199.0 showed a ^3J coupling with the protons of the methylene at δ 2.43, thus completing the valence of the quaternary carbon C-4 at δ 46.7. At this stage, the working hypothesis accounted for a $C_{33}H_{45}O_4$ formula, leaving C_2HO_2 and three valences on the large fragment unoccupied, requiring a second ring.

Structurally speaking, the main difference between the structures of laxifloranone (**2**) and mahureone A (**1**) was the absence of the elements of phloroglucinol, as a triketone or tautomer thereof. Despite the fact that one carbon resonance could not be observed, a bicyclic structure embodying a pyran best fit the spectroscopic observations. The absence of the central carbon atom of the triketone may be due either to keto−enol equilibrium or to unfavorable relaxation parameters in the vicinity of the nonprotonated atoms (eight contiguous quaternary carbon atoms in the structure). From a biosynthetic standpoint, mahureone A (**1**) probably arises from laxifloranone (**2**) through ring opening of the strained nonenolizable 1,3-diketone ring in basic medium or under nucleophile attack followed by ring closure of the intermediate enol (Scheme 1).

Mahureone A (**1**), like laxifloranone (**2**), possesses three asymmetric centers that are not directly linked through vicinal interproton couplings. Comparison of the proton NMR data for the two products proved difficult because the spectra were run in different solvents (pyridine and methanol). In particular, overlap between the protons H-5ax and H-6 precluded direct measurement of their coupling constant, but ROEs were observed between H-5ax and one of the methyl groups attached to C-7. Identification of H-5ax and H-5eq was facilitated by the observation of an ROE between H-5eq and the sharp doublet of H-15. As observed in laxifloranone (**2**), ROEs were detected between the protons of the dihydrocinnamoyl residue and the pair of geminal methyls. Similarities between the ROEs measured for mahureone A (**1**) and NOEs measured for laxifloranone (**2**) led us to propose that the molecules have the same relative (and probably also absolute) configurations, i.e., 4*R**, 6*R**, 27*R**.[11]

Besides **1**, four other related compounds, **3**−**6**, for which the trivial names mahureones B−E are proposed, were isolated; they also inhibited Pol β, albeit to a lesser extent. Mahureone B (**3**) is an isomer of **1** and **2** and has very similar UV, IR, MS, and NMR spectra. The main differ-

Scheme 1. Possible Pathway between Laxifloranone (**2**) and Mahureone A (**1**)



ences in the NMR spectra were linked to the acyl moieties. In compound **1**, one could observe the signals of a 3-methylbutyric acid, but **3** showed the elements of a 2-methylbutyryl chain characterized by a methyl doublet at δ 1.08 and a triplet centered at δ 0.78 ($J \approx 7$ Hz in both cases). The signals of the methylenes in these chains also appeared at slightly different fields (see Table 1). As in compound **1**, two of the carbonyls gave ^3J correlations, one with the acyl chain methyl doublet, i.e., from Me-14 to CO-10, and the other from CH_2-15 to CO-3. The carbon atoms of the C-8, C-9 double bond were detected through correlations with H-15 and H-27, while C-1 and C-2 were observed as broad signals at δ 160.4 and 100.6, respectively; no correlations were observed for these signals as a result of a lack of protons in the immediate vicinity (C-2 was observed owing to the availability of an NMR cryoprobe for this molecule and for compounds **4**, **6**−**9**). Although the side chain in **3** contained an extra asymmetric carbon atom, the molecule appeared as a single diastereoisomer of hitherto unknown configuration.

Compound **4**, mahureone C, has one carbon less than compounds **1** and **3**. The spectra of **1**, **3**, and **4** were very similar, with the most salient differential features for **4** observed in the NMR signals of the acyl side chain, having two nonequivalent three-proton doublets coupling with a methine at δ 3.35. All other signals including the quaternary carbons C-1, -2, -3, and -10 appeared at the expected fields: 161.0, 100.0, 199.3, and 205.9 ppm. The acyl side chain in **4** is thus an isobutyryl moiety instead of the 3- or 2-methylbutyric acid chains found in compounds **1** and **3**.

Compounds **5** and **6**, mahureones D and E, respectively, are isomers and belong to a different series lacking the phenyl nucleus of the dihydrocinnamyl chain. Both showed a molecular ion at *m/z* 551 $[M + Na]^+$ corresponding to the formula $C_{32}H_{48}O_6$. Their ^{1}H and ^{13}C NMR spectra showed that the core of the molecule was formed by the cyclohexene ring substituted by a *gem*-dimethyl group and two isoprenyl chains. The accidental equivalence of the protons of the methylene of the angular isoprenyl unit was absent in these compounds, and these signals appeared as a complex two-proton multiplet centered at 2.46 ppm. A three-proton triplet appeared in the high-field area of the ^{1}H NMR spectrum, part of an *n*-propyl chain, itself coupled to a $CHCH_2$ unit giving resonances in the 2.5−3 ppm zone. An HMBC experiment indicated that the latter element had the same connectivity pattern as the $CHCH_2$ part of the dihydrocinnamic acid moiety. Biogenetically speaking, the cinnamic acid was replaced by a hexenoic acid in mahureones D (**5**) and E (**6**). Complete analysis of the 2D NMR spectra showed that the differences between the two structures arise from the acyl chains as observed in the products mahureones A (**1**) and B (**3**). In compound **5**, the side chain corresponded to a 3-methylbutyric acid, while in **6**, it was a 2-methylbutyric acid. Table 1 summarizes the ^{13}C NMR data for these compounds.

All the mahureones were purified by reversed-phase HPLC on C_{18} columns using gradients of water in acetonitrile with 0.05% TFA added. In several instances, while purifying larger quantities of these compounds, and in particular compound **3**, we noticed the concurrent formation of three new compounds of higher polarity and possessing the same UV spectra as the natural products according to their diode-array detection profiles. The three products, **7**−**9**, showed molecular ions at *m/z* 622, 581, and 699, respectively (positive ESIMS: $[M + H]^+$), and incorporated 59, 18, and 114 additional mass units.

Compound **8**, a hydration product of **3**, showed a single dimethylallyl chain in the COSY experiment, which was located at the angular position (i.e., the isoprenyl part of the lavanduloyl residue was hydrated). Hydration followed Markovnikoff rules, and the methyl carbons gave resonances at 27.6 ppm, while the methyl protons (δ 1.25) showed a three-bond coupling with a quaternary oxygenated carbon at δ 69.9; owing to the asymmetry of the molecule, these signals were slightly nonequivalent.

Compound **9** presented the same general features as compound **8**: occurrence of a single isoprene unit and signals for two nearly equivalent methyl groups at δ 24.2 and 24.5, linked to an oxygenated quaternary carbon atom at δ 89.4. The observation of a sharp quartet at δ 155.0 (J = 40 Hz) in the ^{13}C NMR spectrum suggested the presence of a trifluoroacetate. As could be expected from the effects of trifluoroacetylation, β carbons such as the terminal methyl and methylene 21 were displaced to higher field (ca. 3 ppm).

The last unknown compound, **7**, had an odd molecular ion, which indicated the inclusion of a nitrogen atom. Most portions of their NMR spectra have close resemblances with the NMR spectra of compounds **8** and **9**, in particular the disappearance of the signals for the lavanduloyl isoprene unit and its replacement by a system terminated by a *gem*-dimethyl group, including a quaternary carbon atom. This quaternary carbon gave a resonance at δ 53.2, at a much higher field than observed for compounds **8** and **9**. Comparison of the NMR spectra of compounds **7**−**9** showed the presence of two supplementary elements in **7**: a carbonyl at δ 171.2 and a methyl at δ 22 (δ = 1.93), with both signals showing cross correlations in HMBC experiments. These were included in an acetamido group implanted on quaternary carbon C-22 to account for the composition as determined by mass spectrometry.

The isolation of these three compounds indicates that the starting material **3** is a labile molecule and that when HPLC solvents used to separate this class of compound include traces of acids, care must be exercised during the final isolation step. The dimethylallyl appendage is prone to protonation, which results in the direct formation of tertiary alcohol **8** and of its trifluoroacetate **9**, along with a Ritter reaction product, **7**, formed by the addition of acetonitrile to the double bond. Worthy of note is the selectivity of these reactions, since the other highly similar isoprene unit does not behave similarly, and the absence of neighboring group participation due to the distance between the potentially reactive groups.

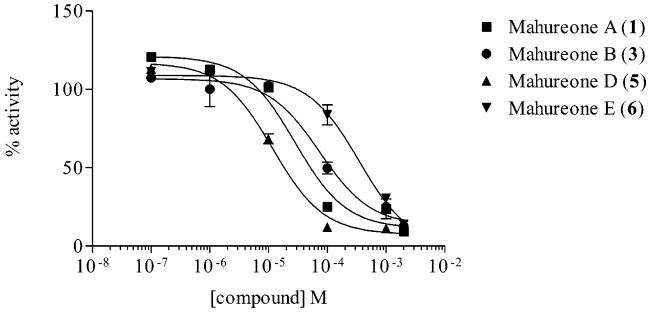


Figure 3. Response of compounds **1** and **3**−**6** on DNA polymerase β activity.

Electron impact gave ions of very low intensity in the higher mass range, with the spectra being dominated by a prominent ion at *m/z* 69 corresponding to the loss of one of the isoprenyl chains; an ion at *m/z* 91 (tropylium) was observed for compound **3**. The use of ion trap electrospray mass spectrometry gave molecular ions and fragments in the positive and negative modes. In the positive mode, the $[M + Na]^+$ ion gave a typical fragmentation with formation of two ions corresponding to the losses of 69 and 128 mass units from the pseudomolecular ion, with the $[M + Na - 69]^+$ ion losing, in turn, 59 mass units. As an example, in compound **1**, these ions were observed at *m/z* 585 $[M + Na]^+$, 516 $[M + Na - 69]^+$, and 457 $[M + Na - 128]^+$; the latter ion finally lost a molecule of water (*m/z* 439). In the negative mode, the fragmentation pattern was different and exhibited losses of 69, 113 (69+44), and 128 mass units. The assignment of these ions to specific fragments of the molecule was rather straightforward, with the loss of the 69 (C_5H_9) fragment arising from the angular dimethyl-allyl chain (loss from the lavanduloyl residue was disregarded owing to a similar pattern in compounds **7**−**9**). It is interesting to note that the efficient loss of 128 mass units in all compounds from the series probably arises from simultaneous, if not concerted, losses of the angular dimethyl-allyl and acetic acid chains to yield a conjugated diene.

Human DNA polymerase β activity was determined by measuring the amount of fluorescein-12-dCTP incorporated in a 60-mer biotinylated oligonucleotide hybridized to a 5′,17-mer synthetic primer to serve as a DNA template. The biotinylated double-strand oligonucleotide was immobilized in a streptavidin-coated microplate. Following the elongation reaction and washing, the fluorescence was then measured. The ddCTP chain terminator was used as a reference compound.[12] Screening of the extract library was carried out on a Beckman automated platform, while the bioguided purification was performed manually with the same protocol. All positive results were further verified by an electrophoresis test in which the length of elongation of the primer was measured.

The IC_{50} value of the ddCTP control molecule was 1.3 μM. The IC_{50} values of **1**, **3**, **5**, and **6** were 28, 73, 12, and 342 μM, respectively (Figure 3). Mahureone C (**4**), compounds **7**, **8**, and **9**, and the methyl ester of mahureone A were found to be inactive. We also found[13] in our case that mahureone A (**1**) inhibited human DNA polymerase β without affecting the replicative ability of nuclear extracts from HeLa human cells, suggesting that it does not interact with the replisome machinery made up by replicative DNA polymerases Pol δ, α, and ϵ and replicative factors RFC and PCNA.[14]

These results suggest that some structural elements are necessary for the activity, particularly the acid and the lavanduloyl isoprenyl side chains (since no activity was seen for the methyl ester and the rearrangement products). There does not seem to be a strong requisite for a particular chain β to the acid function, since compounds **1** and **5** are equally potent. Although the variations around the acyl moiety seem marginal (isopropyl, isobutyl, and 2-butyl chains), they have consequences for activity, and it appears that branching α to the carbonyl greatly decreases the activity. Laxifloranone (**2**) and mahureone A (**1**) share many structural features, and the activity of the former compound on HIV[11] may be due to inhibition of virus reverse transcriptase, an enzyme bearing similarities with the polymerases. Furthermore, we found that 50 μM mahureone A also reduced 2-fold the action of purified HIV reverse transcriptase (data not shown). It is also worth noting that the activity disappears when the carboxyl is blocked.

Recent literature describes a wealth of natural products detected following a polymerase assay-guided purification. Their structures are diverse and include terpenoids,[15] sulfolipids,[16] fatty acids,[17] and phenols including flavonoids.[18] Except for the fact that most of them carry an acid function, none are similar to the present compounds isolated from *M. palustris*. Despite their relative abundance in the plant, tens of grams per kilo, these molecules lack the activity and specificity for further development as adjuncts to a platinum therapy.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer 241 automatic polarimeter. IR spectra were obtained on a Perkin-Elmer Paragon 1000 PC FT-IR spectrometer. NMR were recorded on a Bruker Avance instrument in CD_3OD at 500 MHz for ^1H and 125 MHz for ^{13}C; 2D experiments were performed using standard Bruker programs. Mass spectra were determined on a Bruker Esquire ion trap in the positive or negative electrospray mode. HRMS were measured on a LCT Waters electrospray TOF in the positive ESI mode.

Plant Material. The plant material was collected by one of us (C.M.) in French Guiana (October 1996); identification was performed at the Research Institute for Development Herbarium in Cayenne where an herbarium specimen (No. MFP 3275) is kept.

Extraction and Isolation. Finely ground leaves of *M. palustris* (500 g) were macerated in 5 L of AcOEt for 24 h at room temperature; after filtration, evaporation of the residue yielded 30 g of a waxy residue, which was chromatographed on 950 g of silica gel using a gradient of MeOH in $CHCl_3$ (1 h: pure $CHCl_3$, 1 h: 2% MeOH in $CHCl_3$, 1 h: 1:1 MeOH−$CHCl_3$; and 1 h: pure MeOH); the flow rate was 60 mL/min, and 250 mL fractions were collected. Fractions were pooled according to TLC profiles; compounds **1** and **3**−**6** were in fractions showing spots with R_f values between 0.5 and 0.7 that were eluted with pure chloroform and then with 2% methanol in chloroform. Compounds were obtained in the pure state by reversed-phase preparative HPLC on a C_{18} column [Merck Hibar Lichrospher 100 RP18E (250 × 25 mm)]. The compounds had the following retention times: 39 (**4**), 42 (**1**), 43 (**3**), 46 (**5**), and 47 min (**6**) [Merck Lichrocart Lichrospher 100 RP18E column (125 × 4 mm; flow rate 1 mL/min)]. Approximate yields were 0.86 (**1**), 0.90 (**3**), 0.10 (**4**), 0.70 (**5**) and 1.51 (**6**) g per 100 g of plant.

Mahureone A (1): yellow oil, $[\alpha]_D$ −74° (*c* 1.52, MeOH); UV (acetonitrile) λ_{max} 280 nm; IR ν_{max} (film) 2960, 1737, 1706, 1667, 1556, 1060, 799, 751 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 7.24 (2H, d, J = 7.5 Hz, H-31), 7.18 (2H, t, J = 7.5 Hz, H-32), 7.09 (1H, t, J = 7 Hz, H-33), 5.18 (1H, br t, J = 7 Hz, H-21), 5.05 (1H, br t, J = 7.5 Hz, H-16), 4.08 (1H, br d, J = 10 Hz, H-27), 3.56 (1H, dd, J = 17, 10 Hz, H-28), 2.72 (1H, dd, J = 17, 2 Hz, H-28), 2.68 (1H, dd, J = 13, 7 Hz, H-11), 2.43 (2H, d, J = 7.3 Hz, H-15), 2.35 (1H, dd, J = 13, 7 Hz, H-11), 2.31 (1H, dd, J = 13, 4 Hz, H-20), 2.01 (1H, d, J = 11 Hz, H-5), 1.84

(1H, m, H-20), 1.8 (1H, m, H-12), 1.78 (3H, s, H-23), 1.70 (3H, s, H-24), 1.68 (3H, s, H-18), 1.63 (1H, m, H-6), 1.60 (1H, m, H-5), 1.54 (3H, s, H-19), 1.34 (3H, s, H-25), 1.17 (3H, s, H-26), 0.89 (3H, d, $J = 6.5$ Hz, H-14), 0.87 (3H, d, $J = 6.5$ Hz, H-15); ^{13}C NMR, see Table 1; HRMS *m/z* 585.3181 (calcd for $C_{35}H_{46}O_6Na$ 585.3192).

Mahureone B (3): yellow oil, $[\alpha]_D$ −82° (*c* 1.87, MeOH); IR ν_{max} (film) 3196, 2963, 1735, 1706, 1666, 1555, 1055, 798, 751 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.25 (2H, d, $J = 7$ Hz, H-31), 7.18 (2H, t, $J = 7$ Hz, H-32), 7.08 (1H, t, $J = 7$ Hz, H-33), 5.19 (1H, br t, $J = 7$ Hz, H-21), 5.05 (1H, br t, $J = 7.5$ Hz, H-16), 4.08 (1H, brd, $J = 9.8$ Hz, H-27), 3.57 (1H, dd, $J = 17$, 10 Hz, H-28), 3.28 (1H, m, H-11), 2.72 (1H, dd, $J = 17$, 2 Hz, H-28), 2.44 (2H, d, $J = 8.0$ Hz, H-15), 2.31 (1H, dd, $J = 13$, 5 Hz, H-20), 2.01 (1H, d, $J = 14$ Hz, H-5), 1.84 (1H, m, H-20), 1.78 (3H, s, H-23), 1.70 (3H, s, H-24), 1.68 (3H, s, H-18), 1.62 (1H, m, H-6), 1.57 (1H, m, H-5), 1.53 (3H, s, H-19), 1.45 (1H, m, H-12), 1.33 (3H, s, H-25), 1.23 (1H, m, H-12), 1.17 (3H, s, H-26), 1.08 (3H, d, $J = 6.8$ Hz, H-14), 0.78 (3H, t, $J = 7.3$ Hz, H-13); ^{13}C NMR, see Table 1; HRMS *m/z* 585.3167 (calcd for $C_{35}H_{46}O_6Na$ 585.3192).

Mahureone C (4): yellow oil, $[\alpha]_D$ −41° (*c* 1.13, MeOH); IR ν_{max} (film) 3200, 2962, 1730(sh), 1704, 1668, 1202, 1055, 799, 772 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.25 (2H, d, $J = 7$ Hz, H-31), 7.18 (2H, t, $J = 7$ Hz, H-32), 7.07 (1H, t, $J = 7$ Hz, H-33), 5.18 (1H, br t, $J = 7.6$ Hz, H-21), 5.05 (1H, br t, $J = 7.6$ Hz, H-16), 4.09 (1H, d, $J = 11$ Hz, H-27), 3.56 (1H, dd, $J = 17$, 11 Hz, H-28), 3.35 (1H, m, H-11), 2.72 (1H, dd, $J = 17$, 2.3 Hz, H-28), 2.43 (2H, d, $J = 7.9$ Hz, H-15), 2.31 (1H, dd, $J = 13$, 5 Hz, H-20), 2.00 (1H, d, $J = 10$ Hz, H-5), 1.83 (1H, m, H-20), 1.78 (3H, s, H-23), 1.69 (3H, s, H-24), 1.68 (3H, s, H-18), 1.60 (1H, m, H-6), 1.56 (1H, m, H-5), 1.54 (3H, s, H-19), 1.34 (3H, s, H-25), 1.17 (3H, s, H-26), 1.09 (3H, br d, $J = 6.7$ Hz, H-13), 0.93 (3H, d, $J = 6.4$ Hz, H-12); ^{13}C NMR, see Table 1; HRMS *m/z* 571.3032 (calcd for $C_{34}H_{42}O_6Na$ 571.3036).

Mahureone D (5): yellow oil, $[\alpha]_D$ −92° (*c* 2.3, MeOH); UV (acetonitrile) λ_{max} 225 (sh), 277 nm; IR ν_{max} (film) 2958, 1731, 1705, 1668, 1556, 1099, 1051, 799, 752 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.16 (1H, br t, $J = 7$ Hz, H-21), 5.08 (1H, br t, $J = 7$ Hz, H-16), 3.00 (1H, m, H-11), 2.85 (1H, dd, $J = 16$, 10 Hz, H-28), 2.84 (1H, br m, H-11), 2.69 (1H, br m, H-27), 2.52 (1H, dd, $J = 16$, 3 Hz, H-28), 2.46 (2H, m, H-15), 2.24 (1H, dd, $J = 13$, 5 Hz, H-20), 2.15 (1H, m, H-12), 2.01 (1H, d, $J = 12$ Hz, H-5), 1.77 (3H, s, H-23), 1.74 (1H, m, H-20), 1.70 (3H, s, H-18), 1.67 (3H, s, H-24), 1.63 (2H, m, H-30), 1.56 (3H, s, H-19), 1.52 (2H, m, H-5 and H-6), 1.25 (1H, m, H-31), 1.20 (3H, s, H-25), 1.14 (1H, m, H-31), 1.01 (3H, br d, $J = 6$ Hz, H-14), 0.99 (3H, d, $J = 6$ Hz, H-13), 0.91 (3H, s, H-26), 0.84 (3H, t, $J = 7.3$ Hz, H-32); ^{13}C NMR, see Table 1; HRMS *m/z* 551.3303 (calcd for $C_{32}H_{46}O_6Na$ 551.3349).

Mahureone E (6): yellow oil, $[\alpha]_D$ −87° (*c* 1.63, MeOH); IR ν_{max} (film) 2962, 1731, 1705, 1668, 1556, 1101, 1048, 799, 752 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.16 (1H, br t, $J = 7$ Hz, H-21), 5.09 (1H, br t, $J = 7$ Hz, H-16), 3.79 (1H, m, H-11), 2.85 (1H, dd, $J = 16$, 10 Hz, H-28), 2.70 (1H, m, H-27), 2.51 (1H, dd, $J = 16$, 3 Hz, H-28), 2.46 (2H, m, H-15), 2.24 (1H, dd, $J = 14$, 5 Hz, H-20), 2.01 (1H, d, $J = 11$ Hz, H-5), 1.77 (3H, s, H-23), 1.76 (1H, m, H-20), 1.74 (1H, m, H-12), 1.70 (3H, s, H-18 1.68 (3H, s, H-24), 1.64 (2H, m, H-30), 1.56 (3H, s, H-19), 1.53 (2H, m, H-5 and H-6), 1.48 (1H, m, H-12), 1.24 (1H, m, H-31), 1.22 (3H, t, $J = 6.8$ Hz, H-14), 1.20 (3H, s, H-25), 1.13 (1H, m, H-31), 0.91 (3H, s, H-26), 0.89 (3H, t, $J = 6$ Hz, H-13), 0.83 (3H, t, $J = 7.3$ Hz, H-32); ^{13}C NMR, see Table 1; HRMS *m/z* 551.3336 (calcd for $C_{32}H_{46}O_6Na$ 551.3349).

Compound 7: yellow oil, $[\alpha]_D$ −59° (*c* 1.54, MeOH); IR ν_{max} (film) 3350, 3200, 2962, 1730(sh), 1713, 1660, 1556, 1098, 1054, 799, 751 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.22 (2H, d, $J = 7$ Hz, H-31), 7.18 (2H, t, $J = 7$ Hz, H-32), 7.06 (1H, t, $J = 7$ Hz, H-33), 5.09 (1H, br t, $J = 7.7$ Hz, H-16), 4.08 (1H, br d, $J = 10$ Hz, H-27), 3.58 (1H, dd, $J = 17$, 10 Hz, H-28), 3.26 (1H, m, H-11), 2.71 (1H, br d, $J = 17$ Hz, H-28), 2.48 (2H, m, H-15), 2.04 (1H, br d, $J = 14$ Hz, H-5), 1.93 (3H, s, H-35), 1.68 (3H, s, H-18), 1.64 (1H, m, H-20), 1.54 (3H, s, H-19), 1.47 (1H, m, H-6), 1.34 (3H, s, H-23), 1.31 (3H, s, H-24), 1.28 (3H, s, H-25), 1.15 (3H, s, H-26), 1.08 (3H, br d, $J = 7.3$ Hz, H-14), 0.99 (1H, m, H-20), 0.78 (3H, d, $J = 7.3$ Hz, H-13); ^{13}C NMR, see Table 1; HRMS *m/z* 644.3556 (calcd for $C_{37}H_{51}O_7NNa$ 644.3564).

Compound 8: yellow oil, $[\alpha]_D$ −66° (*c* 1.97, MeOH); IR ν_{max} (film) 3400, 3200, 2960, 2922, 1730, 1713, 1668, 1556, 1099, 1056, 799, 752 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.25 (2H, d, $J = 7$ Hz, H-31), 7.18 (2H, t, $J = 7$ Hz, H-32), 7.07 (1H, t, $J = 7$ Hz, H-33), 5.11 (1H, br t, $J = 7.7$ Hz, H-16), 4.07 (1H, br d, $J = 10$ Hz, H-27), 3.56 (1H, br dd, $J = 17$, 10 Hz, H-28), 3.29 (1H, m, H-11), 2.73 (1H, br d, $J = 17$ Hz, H-28), 2.48 (2H, m, H-15), 2.06 (1H, br d, $J = 12$ Hz, H-5), 1.79 (1H, m, H-20), 1.69 (3H, s, H-18), 1.55 (3H, s, H-19), 1.48 (1H, m, H-6), 1.32 (3H, s, H-25), 1.25 (3H, s, H-23), 1.24 (3H, s, H-24), 1.17 (3H, s, H-26), 1.09 (3H, br d, $J = 6.8$ Hz, H-14), 1.08 (1H, m, H-20), 0.79 (3H, d, $J = 7.5$ Hz, H-13); ^{13}C NMR, see Table 1; HRMS *m/z* 603.3260 (calcd for $C_{35}H_{48}O_7Na$ 603.3298).

Compound 9. Paucity of material precluded recording of IR and $[\alpha]_D$ measurements. ^{1}H NMR (CD_3OD, 500 MHz) δ 7.22 (2H, d, $J = 7.5$ Hz, H-31), 7.17 (2H, t, $J = 7.5$ Hz, H-32), 7.07 (1H, t, $J = 7.5$ Hz, H-33), 5.10 (1H, br t, $J = 7$ Hz, H-16), 4.07 (1H, br d, $J = 10$ Hz, H-27), 3.56 (1H, m, H-28), 3.28 (1H, m, H-11), 2.75 (1H, br d, $J = 17$ Hz, H-28), 2.48 (2H, m, H-15), 2.08 (1H, m, H-21), 2.06 (1H, m, H-5), 1.74 (1H, m, H-20), 1.70 (3H, s, H-18), 1.63 (3H, s, H-23), 1.61 (3H, s, H-24), 1.56 (3H, s, H-19), 1.48 (1H, m, H-6), 1.30 (3H, s, H-25), 1.15 (3H, s, H-26), 1.11 (1H, m, H-20), 1.09 (3H, br d, $J = 6.8$ Hz, H-14), 0.79 (3H, d, $J = 7.5$ Hz, H-13); ^{13}C NMR, see Table 1; HRMS *m/z* 699.3164 (calcd for $C_{37}H_{47}F_3O_8Na$ 699.3121).

Bioassays. Oligonucleotides. Two different oligonucleotides (60-mer and the complementary 3′ end 17-mer)[7a] were synthesized with the PerSeptive Biosystems Expedite system from the Laboratoire de Biologie Moléculaire Eucaryote (CNRS, Toulouse, France) and were purified by precipitation with cold 100% ethanol.

Compounds. Mahureones A−E (**1**, **3**−**6**) and compounds **7**, **8**, and **9** were dissolved in 100% DMSO to afford 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, and 2×10^{-3} M concentrations. Recombinant human DNA polymerase β was from Trevigen.

Assays. The standard reaction mixture contained the following components in a final volume of 50 μL in streptavidin-coated combiplates C8 (Thermolabsystem): 50 mM Tris HCl (pH 8.8), 10 mM $MgCl_2$, 100 mM KCl, 1 mM DTT, 10% glycerol, 25 pmol of biotinylated hybridized oligonucleotide, 0.25 unit of purified DNA polymerase β, and various concentrations of each compound and extract as previously indicated. The reaction started with the simultaneous addition of 10 μM each dATP, dCTP, dTTP, and dGTP and 1 μM fluorescein-12-dCTP. The reaction mixture was incubated for 3.5 h at 37 °C and washed four times with 100 μL of a PBS Tween 0.05% buffer. Then, 50 μL of PBS was added in each well and fluorescence was measured in a Fluostar fluorimeter (BMG). The effect of each compound on the DNA polymerase β activity was determined as a percentage of the DMSO control. These percentages were calculated as followed: % activity = (RFU "tested compound"/RFU DMSO control) × 100 (RFU: relative fluorescence unit). Graph Pad Prism Software was used to calculate nonlinear regression curves of inhibition with the sigmoidal dose−response mode. The concentration of compounds that reduced the signal of the control by 50%, termed the IC_{50} value, was also calculated and used as a measure of the inhibition. Results were reproducible in at least two independent experiments.

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