Vasorelaxant Activity of Diterpenes from *Croton zambesicus* and Synthetic Trachylobanes and Their Structure−Activity Relationships

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The diterpenes previously isolated from the leaves of *Croton zambesicus* were tested to evaluate their vasorelaxant activity on the Wistar rat aorta. Their vasorelaxant effect was compared to a series of synthetic trachylobanes and related polycyclic compounds on KCl- or noradrenaline-induced contractions in order to evaluate structure−activity relationships. We demonstrate the vasorelaxant properties of some pure trachylobane diterpenes at low concentration (IC_{50} < 10 μM) on KCl-induced contractions, but none have a significant effect in noradrenaline-induced contractions. Comparing structures and activity we observed that a C-14 carbonyl group associated with a C-15 hydroxy or ketone function or a C-3 carbonyl associated with a hydroxymethyl group plays an important role in the vasorelaxant activity of trachylobane diterpenes. We also observed that the absolute configuration or the cleavage of the C13−C16 cyclopropane bond does not have a marked effect on the activity. The cytotoxicity of all of these compounds has also been evaluated on HeLa cells in order to verify that the vasorelaxant activity was not correlated with general cytotoxicity.

Croton zambesicus is a tree whose leaves are frequently used in traditional medicine in Benin to treat hypertension, urinary infections, and other diseases.[1] Previous studies reported the isolation of three trachylobanes (compounds **4**, **7**, and **8**), one isopimarane (**5**), a *trans*-phytol (**6**), and two diterpenes (**1** and **2**) (see Chart 1).[2,3] The mixture of **1** and **2** has been shown to inhibit the KCl-induced contraction of male Wistar rat aorta in a concentration-dependent manner with an IC_{50} of about 3.8 μM (1 μg/mL).[4] In the present study we evaluated the vasorelaxant effect of the diterpenes isolated from the leaves of *C. zambesicus* and compared them with several synthetic trachylobanes and some other related polycyclic compounds (compounds **9** to **26**) in the KCl-precontracted aortic ring assay.

Results and Discussion

Diterpenes **1** and **4**−**8** in addition to the mixture of **1** and **2** were previously isolated in our laboratory from the CH_2Cl_2 extract of the leaves of *C. zambesicus*. The isolation procedure and the characterization of these metabolites, as well as the purification of compound **3** from the mixture of **1** and **2**, have been reported previously.[2−4]

The synthetic compounds used in the present vasorelaxant activity study were prepared in enantiomerically pure form following a diastereoselective approach previously developed by some of us for the preparation of trachylobanes and biogenetically related atisane-, beyerane-, and kaurane-type diterpenes from either (*S*)-(+)- or (*R*)-(−)-carvone.[9,12] The following compounds have been described: 15β-hydroxy-*ent*-trachyloban-14-one (**9**),[9] *ent*-trachylobane-14α,15β-diol (**11**),[9] 18-bromo-*ent*-trachylobane-14,15-dione (**14**),[10] 18-hydroxytrachylobane-3,14,15-trione (**17**),[10] 15α-hydroxytrachyloban-14-one (**18**),[9] 15β-iodotrachyloban-14-one (**19**),[9] 15α-hydroxybeyeran-14-one (**28**),[9] *ent*-beyerane-14α,15β-diol (**29**),[11,12] 16βH-atisane-3,14,15-trione (**30**),[11,12] atis-16-ene-3,14-dione (**31**),[11] 19-*nor*-*ent*-trachylob-3-ene-14,15-dione (**32**),[11] and 3β-*tert*-butyldimethylsilyloxy-3,18-*cyclo*-trachylobane-14,15-dione (**33**).[11] The remaining synthetic trachylobanes are new and have been prepared by simple chemical transformations of some of the aforementioned compounds (or their enantiomers). In particular, diketone **10** was readily prepared in high yield by oxidation of the 15-hydroxy group of hydroxyketone **9** with the Jones reagent (Scheme 1).

Trachylobanetrione **20** was obtained by treatment of compound **33** with PTSA in refluxing chloroform, which effected both cyclopropane ring cleavage and simultaneous hydrolysis of the *tert*-butyldimethylsilyloxy group (Scheme 2). Stereoselective reduction of the C-15 carbonyl group of **33** with $NaBH_4$ in a mixture of MeOH and CH_2Cl_2 at low temperature, followed by treatment with PTSA as above, afforded the hydroxytrachylobanedione **21** in an excellent overall yield. This alcohol was readily transformed into the iodide **22** by treatment of the corresponding mesylate with NaI in refluxing acetone.

Compounds **12** and **13** were prepared in the same way as described in the previous paragraph for the preparation of their enantiomers **20** and **21**, respectively, but starting from the enantiomer of compound **33**. As shown in Scheme 1, stereoselective reduction of the 3- and 15-keto groups of **12** with excess $NaBH_4$ afforded the dihydroxytrachylobanedione **15**, in excellent yield.

The remaining compounds, except **16**, and the seco-trachylobanes **23**−**26**, were prepared from the known diketone **32**.[9] Thus, compound **16** was prepared by regio- and stereoselective bromomethoxylation of the C3−C4 double bond of **32** using NBS and MeOH (Scheme 3). Allylic chlorination of **32** using solid CO_2 and calcium hypochlorite occurred with isomerization of the endocyclic C3−C4 double bond to the less substituted exocyclic position, yielding the allyl chloride **24** in good yield. Similarly, allylic bromination of **32** with NBS gave the bromide **23**, which was readily debrominated to give compound **26** by treatment with a DMF solution of $CrCl_2$ and *i*-PrOH as the proton source. The preparation of the allylic alcohol **25** was not possible by direct oxidation of **26** or **32**. However, **25** was prepared very efficiently by a two-step sequence, involving the stereoselective β-epoxidation of the double bond of **32** with *m*-CPBA and subsequent regioselective opening of the oxirane ring with diethylaluminium 2,2,6,6-tetramethylpiperidine (DATMP) in benzene.

For the bioassays, the compounds were dissolved in DMSO and tested at concentrations ranging from 0.3 to 10 μg/mL. The same

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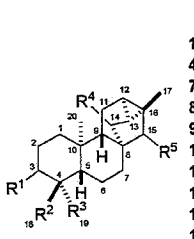
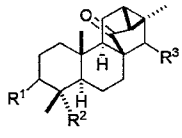
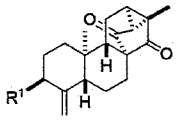
Chart 1. Structures of Natural and Synthetic Compounds Tested for Their Vasorelaxant Activity



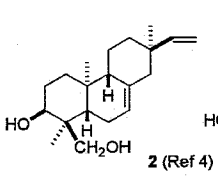
	R^1	R^2	R^3	R^4	R^5	
1	β-OH	CH_2OH	CH_3	H	H	(Ref 4)
4	β-OH	CH_3	CH_3	H	H	(Ref 2)
7	=O	CH_3	CH_3	H	H	(Ref 3)
8	=O	CH_2OH	CH_3	H	H	(Ref 3)
9	H	CH_3	CH_3	=O	β-OH	(Ref 9)
10	H	CH_3	CH_3	=O	=O	(a)
11	H	CH_3	CH_3	α-OH	β-OH	(Ref 9)
12	=O	CH_3	CH_3	=O	=O	(a)
13	=O	CH_3	CH_3	=O	β-OH	(a)
14	=O	CH_2Br	CH_3	=O	=O	(Ref 10)
15	α-OH	CH_3	CH_3	=O	β-OH	(a)
16	β-Br	CH_3	OCH_3	=O	=O	(a)



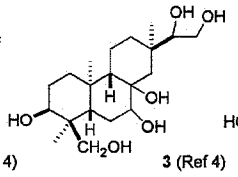
	R^1	R^2	R^3	
17	=O	CH_2OH	=O	(Ref 10)
18	H	CH_3	α-OH	(Ref 9)
19	H	CH_3	β-I	(Ref 9)
20	=O	CH_3	=O	(a)
21	=O	CH_3	α-OH	(a)
22	=O	CH_3	β-I	(a)



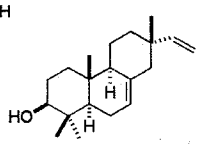
	R^1	
23	Br	(a)
24	Cl	(a)
25	OH	(a)
26	H	(a)



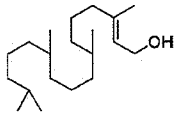
2 (Ref 4)



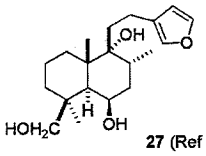
3 (Ref 4)



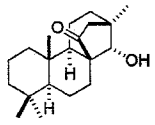
5 (Ref 3)



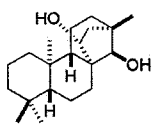
6 (Ref 3)



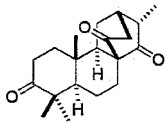
27 (Ref 5)



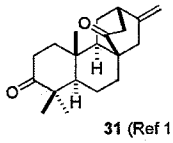
28 (Ref 9,12)



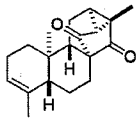
29 (Ref 9,12)



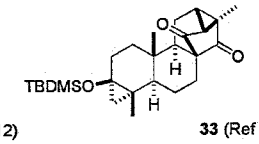
30 (Ref 11,12)



31 (Ref 11,12)

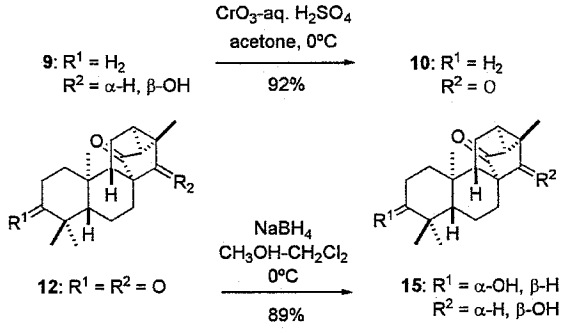


32 (Ref 9,12)

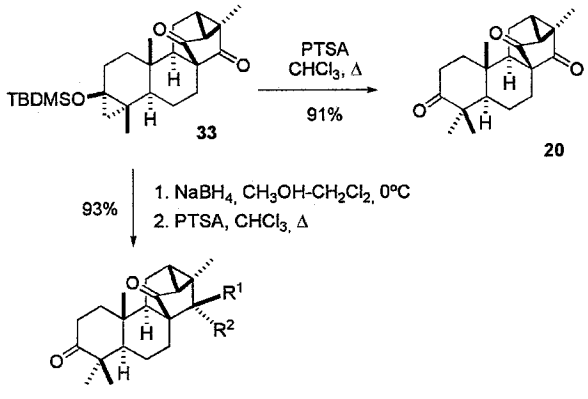


33 (Ref 11,12)

Scheme 1



Scheme 2

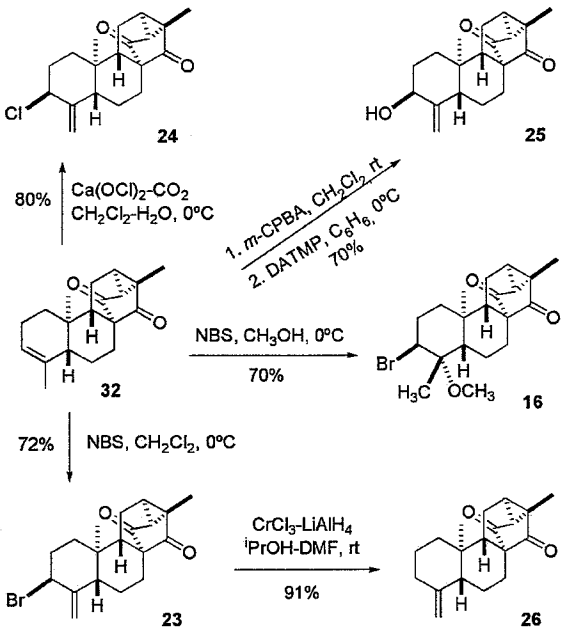


volume of DMSO added to control rings (maximal concentration of DMSO in the organ bath = 0.1%) had no effect on vascular tone. Marrubenol (**27**), a diterpene with known vasorelaxant activity associated with Ca^{2+}-channel antagonist properties, was used as reference compound.[5]

Most of the diterpenes isolated from *C. zambesicus* induced a concentration-dependent relaxation on the KCl-induced contraction at concentrations lower than 10 μg/mL, as shown in Figure 1A, but did not have a significant effect on noradrenaline-induced contraction (data not shown). Their IC_{50} values are given in Table 1, which shows that 18-hydroxy-*ent*-trachyloban-3-one (**8**) is the most potent among the pure compounds, with an IC_{50} = 6.3 μM.

This is the first report showing vasorelaxant activity for a pure trachylobane at low concentration. Although the number of natural trachylobanes tested is too low to establish a reliable structure–

Scheme 3



activity relationship, some comments can be deduced from these data. It seems that the presence of a carbonyl group at C-3 could play an important role in the vasorelaxant activity of this type of natural diterpenes. In fact, diterpene **8**, having a carbonyl group at C-3 and a hydroxymethyl group at C-4, exhibited a much more potent activity than **1**, which has a hydroxyl group at C-3 and a hydroxymethyl group at C-4. However, this is not the only important functional characteristic for high activity since *ent*-trachyloban-3-one (**7**), which also has a carbonyl group at C-3, showed a lower vasorelaxant activity. As deduced from the comparison of the activity of **7** and **8**, the presence of a hydroxymethyl group at C-4 in addition to a carbonyl group at C-3, seems to greatly increase the inhibitory activity of this type of diterpenes on KCl-induced contractions.

In order to further investigate how the nature and location of the substituents at different positions of the trachylobane skeleton affect the vasorelaxant activity, we also tested the synthetic trachylobanes and related polycyclic compounds. Some of them belong to the same enantiomeric series as the natural trachylobanes (the *ent*-series, see below), while others have opposite configuration. They were tested at a concentration of 10 μg/mL (25.4 to 35.2 μM), and the percentages of inhibition of KCl- or noradrenaline-induced contractions were calculated after 40 min of incubation of the rings in the presence of the diterpene. We first observed that none of the synthetic trachylobanes significantly inhibited noradrenaline-induced contractions (data not shown), while some were active on KCl-induced contraction (Table 2). The concentration–response curves for the most active compounds were established by cumulative addition on the plateau of contraction (Figure 1B), and their IC_{50} values were determined (Table 3). Removal of endothelium did not affect the potency of the active compounds (data not shown).

Among the active compounds, diterpenes **26**, **9**, and **10** showed a higher activity than marrubenol (IC_{50} = 10 μM) with IC_{50} values of 3.5, 4.6, and 5.8 μM, respectively, while compounds **18** and **24** were active in the same range. Comparison of the results for the synthetic compounds highlights the importance of the location of some substituents at strategic positions of the trachylobane framework for the vasorelaxant activity.

Influence of Substitution at C-3. Degree of Oxidation at C-3.

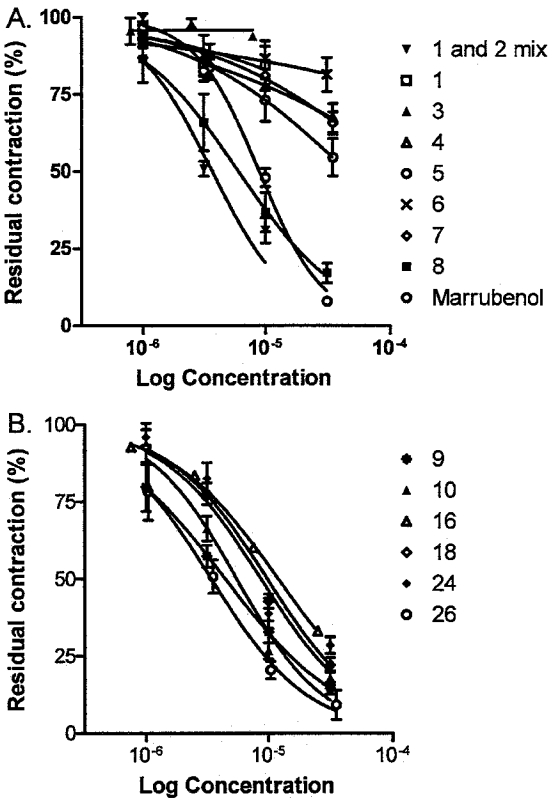


Figure 1. Concentration–response curves of the diterpenes isolated from *C. zambesicus* (A) and of the most active synthetic diterpenes (B) on rat aorta. Effect was measured on endothelium-intact aortic rings precontracted with KCl (100 mM), and compounds were added cumulatively (10^{-6} to 10^{-4} M). Each point represents the mean ± SEM from 3–6 determinations.

Table 1. Vasorelaxant Activity of Diterpenes Isolated from *C. zambesicus* on KCl-Induced Contraction (100 mM)

compound	IC_{50} value (μM)	IC_{50} value (μg/mL)
1	>80	>30
3	>80	>30
4	>80	>30
5	46.2 (42.5 to 50.1)	13.32 (12.28 to 14.45)
6	>80	>30
7	>80	>30
8	6.3 (5.9 to 6.4)	1.92 (1.80 to 1.94)
mix of **1** and **2**	3.8 (3.0 to 5.0)	1.17 (0.91 to 1.52)
marrubenol	10.0 (7.5 to 10.5)	3.36 (2.51 to 3.54)

Each value represents the IC_{50} with the 95% Confidence Interval between brackets

Contrary to the patterns previously observed for the natural trachylobanes, the inhibitory potency of synthetic compounds possessing an additional carbonyl group or a hydroxyl group at C-3 was lower than those lacking this group, e.g., compound **10** vs **12**, **9** vs **13**, **18** vs **21**, **19** vs **22**, or **26** vs **25**.[13] In fact, the more active synthetic compounds (**9**, **10**, or **26**) are C-3 deoxy analogues. It is also observed that the presence of a hydroxy group at C-3 produces a strong detrimental effect on the vasorelaxant activity (**26** vs **25** and **15** vs **9**).

Halogen Substitution at C-3. The presence of a C-3 β-halogen atom in the 19-nor-trachylobane system markedly reduced the vasorelaxant effect. Thus, **23** and **24**, having respectively bromine and chlorine atoms at C-3, are significantly less active than the parent nonhalogenated analogue **26**. It is interesting to note that bromine substitution has a higher detrimental effect on the vasorelaxant activity than chlorine.

Table 2. Inhibitory Effects of Constituents from *C. zambesicus* and Their Synthetic Derivatives on KCl-Induced Contractions in Rat Aortic Rings[a]

compound	residual contraction (%) at 10 μg/mL
mix of **1** and **2**	[b]
1	[b]
3[c]	[b]
4	67.3 ± 4.7 (3)*
5	54.6 ± 6.1 (3)*
6	81.4 ± 5.5 (3)*
7	65.6 ± 3.7 (3)*
8	17.0 ± 3.2 (3)*
9	17.9 ± 2.3 (4)*
10	14.5 ± 2.0 (3)*
11	73.4 ± 1.3 (5)*
12	84.4 ± 1.3 (3)*
13	92.7 ± 1.4 (3)
14	71.8 ± 3.3 (3)*
15	90.3 ± 2.0 (3)
16	33.0 ± 7.1 (6)*
17	93.1 ± 2.6 (3)
18	22.1 ± 2.4 (4)*
19	37.4 ± 9.3 (3)*
20	91.4 ± 2.6 (3)
21	90.0 ± 3.0 (3)
22	95.7 ± 1.8 (3)
23	65.5 ± 0.9 (3)*
24	28.5 ± 2.7 (4)*
25	83.9 ± 4.5 (3)*
26	9.2 ± 2.7 (3)*
28	40.7 ± 1.7 (3)*
29	52.2 ± 1.4 (3)*
30	87.9 ± 3.9 (3)
31	60.6 ± 17.9 (3)*
32	[c]
33	[c]
marrubenol (**27**)	7.9 ± 0.7 (5)*

[a] Each value represents the mean ± SEM of (n) determinations. *Significantly different from the control: $p < 0.05$. [b] Maximal concentration realized in organ bath: 3 μg/mL. [c] Obtained from the hydroxylation reaction of the mixture of **1** and **2** (see Experimental Section). [d] Not determined because of small amount.

Table 3. Vasorelaxant Activity of the Synthetic Compounds on KCl-Induced Contraction (100 mM)[a]

compound	IC_{50} value (μM)	IC_{50} value (μg/mL)
9	4.65 (4.6 to 4.7)	1.41 (1.40 to 1.43)
10	5.8 (4.8 to 7.0)	1.75 (1.46 to 2.10)
16	12.3 (12.1 to 12.5)	4.86 (4.77 to 4.96)
18	9.1 (8.2 to 10.1)	2.75 (2.49 to 3.05)
24	9.75 (7.6 to 12.4)	3.11 (2.44 to 3.95)
26	3.5 (3.4 to 3.6)	1.01 (0.97 to 1.04)

[a] Each value represents the IC_{50} with the 95% confidence interval between parentheses.

Influence of Substitution or Hybridation at C-4. Functionalization of the C-18 Position. In sharp contrast with what was observed for the natural trachylobanes with a carbonyl group at C-3, the hydroxylation of the equatorially oriented methyl group at C-4 in the trachylobanes oxygenated at C-14 and C-15 does not significantly influence the activity, e.g., compound **17** vs **20**. However, the activity of these two compounds is too low to draw a definitive conclusion. A small positive effect is observed with the bromination of this position, as deduced from comparison of the data for **12** and **14** in Table 2.

Methylidene Group at C-4. The conformational changes of the A ring as a consequence of the tetrahedral to planar geometry conversion at C-4 does not result in a decrease of the activity of compounds oxygenated at C-14 and C-15. On the contrary, a comparison of the IC_{50} values of compounds **10** and **26** reveals that this modification has a significant positive effect (**10** is about 1.6-fold less active than **26**, the most active of all the compounds studied).

Influence of Substitution at C-14 and C-15. Compound **10**, having carbonyl groups at C-14 and C-15, and **9**, having a carbonyl group at C-14 and a hydroxy group at C-15, were more active than **11**, which has a hydroxy group at C-14 and C-15. The strong vasorelaxant activities of compounds **9** and **10** support the idea that a carbonyl group at C-14 may be important for the activity. However, the positive effect associated with the presence of the carbonyl group at C-14 seems to be suppressed by the presence of another carbonyl at C-3, as inferred from comparison of the data for **9** and **13**, or **10** and **12**. On the other hand, replacement of the oxygenated function at C-15 by a less polar group, like iodine, produces a decrease of activity (**18** vs **19**).

From these results we conclude that a carbonyl group at C-14 associated with a hydroxy or ketone at C-15 or a carbonyl at C-3 associated with a hydroxymethyl group at C-4 seems to play a decisive role in the vasorelaxant activity of trachylobane diterpenes. Thus, the presence of a nonpolar site at one end of the molecule and an oxygenated more polar site with at least one ketone and another polar substituent at the other end seems important for the activity.

Influence of the Absolute Configuration: Trachylobane vs *ent*-Trachylobane Skeleton. Trachylobane diterpenes are characterized by a pentacyclic carbon skeleton and a tricyclo[3.2.1.$0^{2,7}$]-octane system for rings C, D, and E. They are named trachylobanes when the absolute configuration at C-5, C-9, and C-10 is the same as in steroids. On the contrary, when the configurations at these positions are inversed compared to steroids, they are named *ent*-trachylobanes. It is important to underline at this point that most if not all of the natural trachylobanes of known absolute configuration belong to the *ent*-series. We observed a small difference in the vasorelaxant activity of the enantiomeric trachylobanes **20** and **12** or **9** and **18**. In addition neither **13**, from the *ent*-series, nor **21**, from the normal series, exhibited significant vasorelaxing activity.

Other Structural Modifications. It was interesting to examine how the cleavage of the tricyclo[3.2.1.$0^{2,7}$]octane moiety, characteristic of the trachylobane skeleton, could affect the vasorelaxant activity. We thus determined the inhibitory effect on KCl-induced contraction of rat aortic rings of compounds **28** to **31**. The first two possess a beyerane skeleton that results from the formal cleavage of the C12−C13 bond of the trachylobane system, while **30** and **31** have an atisane framework that results from the cleavage of the C13−C16 cyclopropane bond. Cleavage of the C13−C16 bond does not result in a modification of the activity of the compounds, as deduced by comparison of the data for compounds **30** and **20**. However, rupture of the C12−C13 bond leads to an important loss of activity: **28** is about 50% less active than trachylobane **18**. These results are consistent with the change in the geometry of the molecule as a consequence of the opening of the cyclopropyl moiety. Thus, the cleavage of the C13−C16 cyclopropyl bond does not produce an important modification of the geometry of the C ring, which in both the initial trachylobane system and the resulting atisane framework adopts a boat conformation, while cleavage of the C12−C13 bond leads to a beyerane skeleton in which the C ring adopts a chair conformation.

However, this is not observed in the *ent*-series. In this case, the cleavage of the C12−C13 bond induced a small positive effect on the vasorelaxant activity, as evidenced by comparison of the activity of **29** and **11**. It is important to note that **29** has a hydroxy group at C-14, while **28** has a carbonyl group at this position. Therefore, cleavage of the C12−C13 bond has a positive or a negative effect depending on the presence of a hydroxy or a carbonyl group at C-14 and/or the absolute configuration (**28** and **18** do not belong to the *ent*-series).

In our previous work, we described the cytotoxic activities of diterpenes isolated from *Croton zambesicus* on HeLa cells.[2,3] In order to have a general indication of the potential toxicity of these compounds, we also evaluated the cell viability of the synthetic

Table 4. Cytotoxic Activity on HeLa Cells of Diterpenes from *C. zambesicus* and Synthetic Diterpenes[a]

compound	IC_{50} (μM)	IC_{50} (μg/mL)
mix of **1** and **2**	81.8 ± 14.8	24.9 ± 4.5
1	64 ± 7.2	19.5 ± 2.2
3	[b]	[b]
4	25.3 ± 6.6	7.3 ± 1.9
5	87.8 ± 11.4	25.3 ± 3.3
6	46.6 ± 4.4	13.8 ± 1.3
7	33.5 ± 5.6	9.6 ± 1.6
8	40.4 ± 6.9	12.2 ± 2.1
9	137.6 ± 4.9	41.6 ± 1.5
10	118.3 ± 3.6	35.5 ± 1.1
11	>150	>75
12	>150	>75
13	>150	>75
14	>150	>75
15	>150	>75
16	>150	>75
17	[b]	[b]
18	>150	>75
19	150.4 ± 6.1	62 ± 2.5
20	>150	>75
21	>150	>75
22	>150	>75
23	>150	>75
24	>150	>75
25	>150	>75
26	128.4 ± 4.2	36.5 ± 1.2
27	[b]	[b]
28	152.8 ± 4.9	46.5 ± 1.5
29	>150	>75
30	>150	>75
31	>150	>75
32	124.9 ± 1.8	35.5 ± 0.5
33	>150	>75
camptothecine	0.5	0.174

[a] Each value represents the mean ± SEM. [b] Not determined.

diterpenes by MTT (Sigma) colorimetric assay on HeLa cells. The IC_{50} values are summarized in Table 4. Tested compounds showed a large range of cytotoxic potency, the most potent being **4** and **7**, but we observed that there was no relation between cytotoxic and vasorelaxant properties and that several vasorelaxant molecules are not cytotoxic at the maximum tested dose.

In conclusion, our results demonstrated for the first time the high vasorelaxant activity of some trachylobane diterpenes. Furthermore, we can propose some structure–activity relationships. For compounds lacking the C-14 ketone function, the presence of both a C-3 carbonyl and a C-4 hydroxymethyl group greatly increased the activity (natural compounds). In the absence of a substituent at C-3, the compounds having a C-14 carbonyl group associated with a C-15 carbonyl or hydroxy group showed a higher activity than the compounds having a C-14 hydroxy group. Observations for synthetic compounds are summarized in Figure 2.

Experimental Section

General Experimental Procedures. All melting points were determined using a Kofler hot-stage apparatus and are uncorrected. Optical rotations were determined using a 5 cm path length cell. $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR spectra were recorded in $CDCl_3$ at 300 or 400 MHz, and ^{13}C NMR spectra at 75 or 100 MHz. ^{1}H NMR spectra were referenced to residual $CHCl_3$ (δ 7.26) and ^{13}C NMR spectra to the central component of the $CDCl_3$ triplet at δ 77.0. Carbon substitution degrees were established by DEPT pulse sequences. A combination of COSY, HMQC, and NOE experiments was utilized when necessary for the assignment of ^{1}H and ^{13}C chemical shifts. IR spectra were measured as KBr pellets or liquid films. Mass spectra were obtained by electron impact (EI) at 70 eV. Column chromatography refers to flash chromatography and was performed on Merck silica gel 60, 230–400 mesh. Analytical TLC was performed on precoated silica gel 60 F_{254} plates (Merck), and detection was achieved by spraying with H_2SO_4–anisaldehyde, followed by heating 5 min at 105 °C. High-speed counter-current chromatography was performed on an HSCCC Kromaton III, SEAB. An Omnifit glass column (OM 6427 15 × 750 mm) packed with Lichroprep Si 60 (15–25 μM, Merck) was used for MPLC. All operations requiring anhydrous conditions and/or involving air-sensitive reagents were performed under an inert atmosphere of dry argon using syringes, oven-dried glassware, and freshly distilled and dried solvents.

Plant Material. The aerial parts of *C. zambesicus* were collected in the surroundings of Cotonou (Benin) and identified by Prof. V. Adjakidje (Université d'Abomey-Calavi, Benin). A voucher specimen has been deposited at the herbarium of the Belgian National Botanical Garden at Meise (BR S.P. 848.108).

Extraction and Isolation of Compounds 1 and 4–8 and the Mixture of 1 and 2. Powdered, air-dried leaves (580 g) of *C. zambesicus* were macerated (1.8 L) during 48 h in CH_2Cl_2 and then extracted by percolation (3.2 L) at room temperature. Five grams of the extract was fractionated by HSCCC using the two-phase solvent system heptane–MeCN–CH_2Cl_2 (10:7:3) in the descending mode, giving 21 fractions. The mixture of **1** and **2** was isolated from fraction 3 by successive MPLC. The isolation of pure **1** and hydroxylated pimarane **3** was possible only after hydroxylation of the mixture of **1** and **2** with OsO_4 (0.1 M) at room temperature followed by preparative TLC. Successive MPLC of fractions 20, 9, 14, and 6 gave, respectively, compounds **4** (10 mg), **5** (14 mg), **6** (8 mg) and **7** (25 mg), both from fraction 14, and **8** (20 mg).

Measurement of Aorta Contraction. Male Wistar rats weighing 200 to 300 g were sacrificed by decapitation, and the descending thoracic aorta was isolated, cleaned, and cut in rings (2 mm length). Aortic rings were suspended under a resting tension of 20 mN in 12.5 mL organ baths filled with a physiological solution (composition (mM): NaCl, 122; KCl, 5.9; $NaHCO_3$, 15; $MgCl_2$, 1.25; $CaCl_2$, 1.25; glucose, 11) maintained at 37 °C and bubbled with a gas mixture of 95% O_2 and 5% CO_2. Contractile responses were measured as described previously.[4]

Preparations were equilibrated for 60 min before initiating the experimental procedures. Contractions were evoked by changing the physiological solution in the bath to a depolarizing solution (composition (mM): NaCl, 27; KCl, 100; $NaHCO_3$, 15; $MgCl_2$,1.25; $CaCl_2$, 1.25; glucose, 11) or by adding noradrenaline (10^{-6} M). After washing, the muscle was preincubated for 30 min in the presence of the compounds, and a second contraction was evoked in the continuous presence of the compounds to be tested or the same volume of solvent. The amplitude of contraction evoked in the presence of the tested compound was compared to the response obtained in its absence. Stock solutions were prepared by dissolving in DMSO at a concentration of 10 mg/mL, and compounds were tested at 10 μg/mL on KCl- and noradrenaline-induced contractions. Some compounds were tested at lower concentrations because of their low water solubility. The highest concentration that could be realized in the organ bath for these compounds was 3 μg/mL. To calculate the IC_{50} for the most active compounds, in a second set of tests they were added cumulatively on the plateau of contraction induced by KCl. Selected diterpenes possessing a low water solubility were tested at concentrations from 0.3 to 3 μg/mL, and other selected compounds were tested from 0.3 to 10 μg/mL. For the assays, marrubenol, used as reference compound, was dissolved in DMSO.

Cell Culture and Assessment of Cytotoxicity. Cell viability was determined by the MTT (Sigma) method on a human cervix carcinoma cell line (HeLa) as was described previously.[2,3] The relative absorbances were expressed as percentage of the control, and camptothecin (Sigma) was used as positive control.

Data Analysis and Statistics. The response of aortic rings was expressed as percentage of the initial contraction to KCl (100 mM). Concentration–response curves were analyzed to give the concentration producing 50% inhibition of the maximal contractile response by sigmoidal curve-fitting analysis. All results are expressed as means ± the standard error of the mean (SEM). IC_{50} values are expressed as mean values with their 95% confidence interval. Differences in the concentration–response curves were analyzed by two-way analysis of variance, followed by the Dunett test with a criterion set for statistical significance at $p < 0.05$.

Preparation of New Synthetic Trachylobane-Type Compounds. Experimental procedures for the preparation and appropriate structural

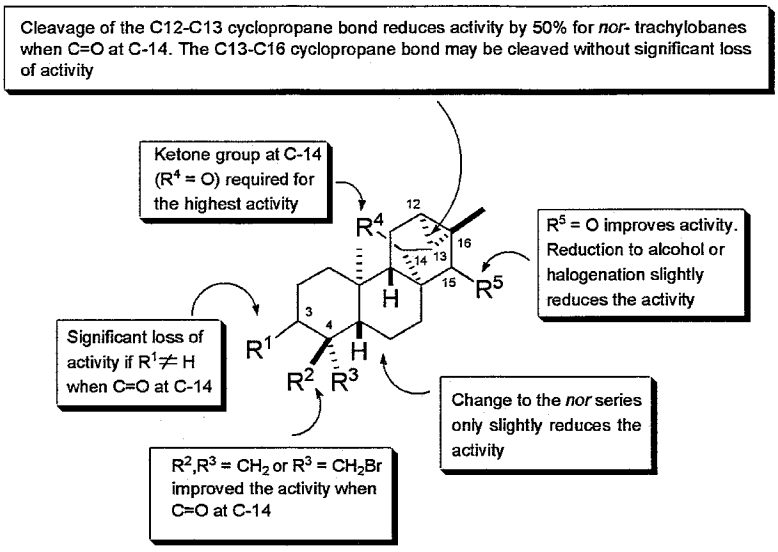


Figure 2. Schematic SAR model for the vasorelaxant activity of synthetic trachylobane diterpenes.

characterization data for compounds **10**, **12**, **13**, **15**, **16**, **20**, **21**, **22**, **23**, **24**, **25**, and **26** are given below.

***ent*-Trachylobane-14,15-dione (10).** A solution of compound **9**[9] (40 mg, 0.132 mmol) in acetone (3 mL) was cooled to 0 °C and dropwise treated with a solution of Jones reagent[14] (ca. 0.5 mL). After a few minutes the reaction mixture was poured into 20 mL of H_2O and extracted with ether. The organic layer was washed successively with H_2O, 5% aqueous HCl solution, and brine and dried over $MgSO_4$. The residue was chromatographed, using hexane−EtOAc (9:1) as eluent, to give compound **10** (37 mg, 92%) as a solid: mp 147−148 °C (from $CHCl_3$); $[\alpha]_D^{29}$ −37 (*c* 1.2, $CHCl_3$); IR (KBr) ν_{max}2909, 2868, 1752, 1696, 1454, 1383, 1137, 1019, 958, 896 cm^{-1}; ^1H NMR (300 MHz) δ 2.49 (1H, ddd, J = 7.7, 2.3, 2.3 Hz, H-12), 2.58 (1H, d, J = 8.1 Hz, H-13), 2.08 and 1.96 (1H each, two m, H_2-11), 1.99 (1H, dd, J = 6.3, 6.3 Hz, H-9), 1.88 and 1.61 (1H each, two m, H_2-1), 1.71 and 1.62 (1H each, two m, H_2-1), 1.61 (2H, m, H-2), 1.43 (1H, m, H-3α), 1.42 and 0.79 (1H each, two m, H_2-1), 1.39 (3H, s, Me−C_{16}), 1.14 (1H, ddd, J = 13.0, 13.0, 3.9 Hz, H-3β), 0.89 (3H, s, Meα-C_4), 0.84 (3H, s, Meβ-C_4), 0.84 (3H, s, Me-C_{10}), 0.75 (1H, m, H-5); ^{13}C NMR (75 MHz) δ 207.13 (C_{15}), 206.67 (C_{14}), 61.09 (C_9), 56.14 (C_8), 54.05 (C_5), 48.89 (C_{16}), 46.88 (C_{13}), 43.54 (C_{12}), 42.72 (C_3), 39.23 (C_1), 38.93 (C_{10}), 33.22 (C_{19}), 32.87 (C_4), 21.86 (C_7), 21.55 (C_{18}), 18.03 (C_6), 19.16 (C_{11}), 18.03 (C_2), 12.80 (C_{17}), 13.57 (C_{20}); EIMS *m/z* (%) 300 (M^+, 100), 285 (20), 163 (94), 138 (61), 135 (46), 123 (90), 91 (66); HREIMS *m/z* 300.2082 (calcd for $C_{20}H_{28}O_2$ 300.2089).

Trachylobane-3,14,15-trione (20) and *ent*-Trachylobane-3,14,15-trione (12). A solution of compound **33**[11,12] (80 mg, 0.19 mmol) and PTSA (46.5 mg, 0.23 mmol) in $CHCl_3$ (4 mL) was refluxed for 3 h, cooled to room temperature, and then poured into H_2O and extracted with ether. The organic layer was washed with 5% aqueous $NaHCO_3$ solution and brine and dried over $MgSO_4$. The residue obtained after evaporation of the solvent was chromatographed, using hexane−EtOAc (7:3) as eluent, to give the trachylobanetrione **20** as a white solid (54 mg, 91%): mp 138−139 °C (from $CHCl_3$); $[\alpha]_D^{29}$ +10 (*c* 1.2, $CHCl_3$); IR (KBr) ν_{max} 2960, 2934, 2863, 1757, 1700, 1454, 1362, 1234, 901, 860, 814 cm^{-1}; ^1H NMR (300 MHz) δ 2.58 (1H, d, J = 7.9 Hz, H-13), 2.49 (1H, ddd, J = 7.9, 2.3, 2.3 Hz, H-12), 2.49 (1H, m, H-2α), 2.23 (1H, ddd, J = 15.8, 5.3, 3.4 Hz, H-2β), 2.15 (1H, ddd, J = 14.7, 12, 2.4 Hz, H-11α), 1.94 (1H, m, H-11β), 1.94 (1H, dd, m, H-9), 1.75 and 1.58 (1H each, two m, H_2-6), 1.65 (2H, m, H_2-7), 1.60 (1H, m, H-1α), 1.38 (3H, s, Me-C_{16}), 1.25 (1H, ddd, J = 13.5, 13.5, 5.3 Hz, H-1β), 1.13 (1H, dd, J= 12.1, 2.3 *Hz*, H-5), 1.03 (3H, s, Meβ-C_4), 0.99 (3H, s, Meα-C_4), 0.96 (3H, s, Me-C_{10}); ^{13}C NMR (75 MHz) δ 215.41 (C_3), 206.56 (C_{14}), 205.86 (C_{15}), 59.67 (C_9), 55.36 (C_8), 53.89 (C_5), 49.09 (C_4), 47.48 (C_{16}), 46.88 (C_{13}), 43.15 (C_{12}), 38.22 (C_{10}), 37.69 (C_1), 33.83 (C_2), 25.42 (C_{18}), 21.64 (C_{19}), 21.62 (C_7), 19.11 (C_{11}), 18.84 (C_6), 12.87 (C_{20}), 12.69 (C_{17}); EIMS *m/z* (%) 314 (M^+, 100), 285 (25), 229 (15), 187 (17), 163 (31), 135 (21), 109 (23), 91 (24); HREIMS *m/z* 314.1883 (calcd for $C_{20}H_{26}O_3$ 314.1882).

The enantiomeric *ent*-trachylobane-3,14,15-trione (**12**) was synthesized in the same way as **20**, but starting from the enantiomer of **33**. The spectroscopic and physical properties are identical to that of **20** with the exception of the sign of the optical rotation.

15α-Hydroxytrachylobane-3,14-dione (21) and 15β-hydroxy-*ent*-trachylobane-3,14-dione (13). A solution of diketone **33**[11,12] (11 mg, 0.026 mmol) in a 1:1 mixture of MeOH−CH_2Cl_2 (0.8 mL) was cooled to 0 °C, and $NaBH_4$ (4 mg, 0.106 mmol) was added in small portions over a period of 30 min. Stirring was continued for 15 min at the same temperature, and then the reaction mixture was quenched with H_2O and stirred for a few minutes until the evolution of hydrogen ceased. The reaction mixture was diluted with H_2O and extracted with ether. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the solvent left a residue that was filtered through a short pad of silica gel, using hexane−EtOAc (7:3) as eluent, to give a solid residue (10.2 mg).

A solution of the product obtained above and PTSA (5.7 mg, 0.028 mmol) in $CHCl_3$ (1 mL) was refluxed for 3 h. Workup as above for **20/12** and purification by chromatography, using hexane−EtOAc (7:3) as eluent, afforded the hydroxytrachylobanedione **21** as a white, amorphous solid (6.7 mg, 93%): mp 192.0−193.5 °C (from hexane−benzene); $[\alpha]_D^{26}$ −16 (*c* 0.37, $CHCl_3$); IR (KBr) ν_{max} 3399, 2945, 2907, 2841, 1716, 1689, 1425, 1405, 1399, 1088, 1022, 908, 634 cm^{-1}; ^1H NMR (300 MHz) δ 3.63 (1H, s, H-15), 2.52 (1H, ddd J = 15.6, 13.5, 6.2 Hz, H-2α), 2.36 (1H, ddd, J = 15.6, 5.1, 3.1 Hz, H-2β), 20.12 (1H, m, H-11), 2.04 (1H, dd, J = 14.6, 4.3 Hz, H-9), 1.81 (1H, m, H-12), 1.85 (1H, m, H-7), 1H, m, H-12), 1.78 (1H, m, H-11′), 1.76 (1H, m, H-6), 1.74 (1H, m, H-13), 1.62 (1H, m, H-6′), 1.60 (1H, m, H-1), 1.29 (1H, m, H-7′), 1.35 (3H, s, Me-C_{16}), 1.41 (1H, m, H-1′), 1.17 (1H,dd, J = 12.6, 2.5 Hz, H-5), 1.04 (3H, s, Meβ-C_4), 0.99 (3H, s, Meα-C_4), 0.91 (3H, s, Me-C_{10}); ^{13}C NMR (75 MHz) δ 216.67 (C_3), 211.81 (C_{14}), 76.74 (C_{15}), 54.63 (C_5), 49.79 (C_8), 48.73 (C_9), 47.67 (C_4), 39.92 (C_{13}), 37.68 (C_1), 37.50 (C_{16}), 37.04 (C_{10}), 34.85 (C_{12}), 34.04 (C_2), 27.64 (C_7), 25.46 (C_{18}), 21.60 (C_{19}), 19.74 (C_6), 18.82 (C_{11}), 17.88 (C_{17}), 13.30 (C_{20}); EIMS *m/z* (%) 317 (M^+, 20), 316 (M^+, 100), 298 (17), 259 (15), 243 (18), 213 (36), 171 (14), 119 (15); HREIMS *m/z* 316.2016 (calcd for $C_{20}H_{28}O_3$ 316.2038).

The enantiomeric 15β-hydroxy-*ent*-trachylobane-3,14-dione (**13**) was synthesized in the same manner as **21** but starting from the enantiomer of **33**. The spectroscopic data of **13** are identical to those of **21** with the exception of the sign of the optical rotation.

3α,15β-Dihydroxy-*ent*-trachyloban-14-one (15). A solution of compound **12** (25 mg, 0.079 mmol) in a 1:1 mixture of CH_2Cl_2−CH_3-OH (3 mL) was cooled to 0 °C, and $NaBH_4$ (12 mg, 0.32 mmol) was added in small portions during 1 h. The mixture was allowed to warm to rt and then treated with acetone to destroy excess $NaBH_4$. After 15 min the mixture was poured into H_2O and extracted with EtOAc. The combined organic extracts were washed with H_2O and brine and dried over $MgSO_4$. The residue obtained after evaporation of the solvent was

purified by chromatography, using hexane−EtOAc (6:4) as eluent, to afford the diol **15** (21 mg, 89%) as a white solid: mp 172−174 °C (from MeOH); $[\alpha]_D^{29}$ −23 (*c* 1.4, $CHCl_3$); IR ν_{max} (KBr) 3486, 3450, 1750, 1440, 1335, 1010, 930, 900 cm^{-1}; ^1H NMR (300 MHz) δ 3.58 (1H, s, H-15), 3.19 (1H, dd, J = 10.9, 4.6 Hz, H-3), 2−1.4 (2H, m, H_2-6), 2−1.4 (2H, m, H_2-11), 1.95 (1H, m, H-9), 1.87 (1H, m, H-12), 1.80 (1H, m, H-13), 1.80 and 1.20 (1H each, two m, H_2-7), 1.50 (2H, m, H_2-7), 1.40 and 0.90 (1H each, two m, H_2-1), 1.34 (3H, s, Me-C_{16}), 0.95 (3H, s, Meβ-C_4), 0.74 (3H, s, Meα-C_4), 0.71 (3H, s, Me-C_{10}), 0.65 (1H, m, H-5); ^{13}C NMR (75 MHz) δ 211.94 (C_{14}), 78.91(C_3), 76.91 (C_{15}), 53.83 (C_5), 49.95 (C_8), 49.50 (C_9), 39.89 (C_{13}), 38.68 (C_4), 37.29 (C_{16}), 37.22 (C_{10}), 37.03 (C_1), 34.91 (C_{12}), 27.98 (C_7), 27.93 (C_{18}), 26.75 (C_2), 18.71 (C_6), 18.71 (C_{11}), 17.92 (C_{17}), 15.58 (C_{19}), 14.04 (C_{20}); EIMS *m/z* (%) 318 (M^+, 8), 300 (24), 285 (4), 261 (10), 105 (11), 135 (21), 91 (15), 86 (61), 84 (100); HREIMS *m/z* 318.2200 (calcd for $C_{20}H_{30}O_3$ 318.2195).

18-Bromo-4α-methoxy-19-*nor-ent*-trachylobane-14,15-dione (16). NBS (39 mg, 0.22 mmol) was added to a solution of compound **32**[9] (50 mg, 0.18 mmol) in MeOH (3 mL) at 0 °C. After stirring for 45 min, the reaction mixture was poured into 50 mL of diethyl ether and washed successively with H_2O and brine. The organic phase was dried over $MgSO_4$, and the residue left after evaporation of the solvent was purified by chromatography, using hexane−EtOAc (6:4) as eluent, to afford the dione **16** (50 mg, 70%) as a white solid: mp 159−162 °C (from MeOH); $[\alpha]_D^{29}$ +34 (*c* 0.5, $CHCl_3$); IR ν_{max}/cm^{-1} (KBr) 2975, 2919, 1757, 1700, 1449, 1424, 1372, 1122, 1075, 937; ^1H NMR (300 MHz) δ 4.45 (1H, dd, J = 2.5, 2.5 Hz, H-3), 3.11 (3H, s, OCH_3-C_4), 2.56 (1H, d, J = 7.9 Hz, H-13), 2.47 (1H, ddd, J = 7.9, 2.6, 2.6 H-12), 2.23 and 1.66 (1H each, two m, H_2-2), 2.1 (1H, m, H-11α), 2.07 (1H, m, H-5), 1.80−1.50 (2H, m, H_2-6), 1.93 (1H, ddd, J = 11.9, 4.7, 2.7 Hz, H-11β), 1.75 (2H, m, H_2-7), 1.42 (1H, m, H-1β), 1.42 (1H, m, H-5), 1.39 (3H, s, Me-C_{16}), 1.31 (3H, s, Meβ-C_4), 0.92 (3H, s, Me−C_{10}), 0.91 (1H, ddd, J = 13.2, 3.5, 3.5 Hz, H-1α); ^{13}C NMR (75 MHz) δ 206.80 (C_{14}), 206.40 (C_{15}), 77.37 (C_4), 59.79 (C_9), 58.05 (C_3), 55.89 (C_8), 49.13 (OCH_3), 48.99 (C_{16}), 47.36 (C_{13}), 46.92 (C_5), 43.26 (C_{12}), 38.62 (C_{10}), 32.94 (C_1), 26.29 (C_2), 23.80 (C_{18}), 21.24 (C_7), 18.92 (C_{11}), 16.96 (C_6), 12.85 (C_{17}), 12.83 (C_{20}); EIMS *m/z* (%) 396 (M^{2+}, 42), 394 (M^+, 44), 364(8), 362 (7), 315 (15), 273 (100), 241 (11), 85 (74); HREIMS *m/z* 394.1134 (calcd for $C_{20}H_{27}O_3Br$ 394.1144).

15β-Iodotrachylobane-3,14-dione (22). Et_3N (20 μL, 0.143 mmol) and MsCl (10 μL, 0.11 mmol) were added to a solution of hydroxyketone **21** (10 mg, 0.03 mmol) in CH_2Cl_2 (2.3 mL) at 0 °C. After stirring at rt for 2 h, the mixture was diluted with diethyl ether, washed successively with 0.5 M HCl, 5% aqueous $NaHCO_3$ solution, and brine, and dried over $MgSO_4$. Evaporation of the solvent under reduced pressure at rt afforded a yellowish residue of crude mesylate (15 mg), which was used in the subsequent step without further purification. The mesylate was dissolved in a 10% solution of NaI in dry acetone (1 mL), and the mixture was heated at 40 °C for 2 h. The reaction mixture was cooled to rt, poured into H_2O, and extracted with hexane. The combined organic phases were washed with dilute aqueous $Na_2S_2O_3$ solution and H_2O, dried over $MgSO_4$, and filtered, and the solvent was evaporated under vacuum. Purification by column chromatography, using hexane−EtOAc (9:1) as eluent, afforded iodoketone **22** (10.8 mg, 85% for the two steps) as a white, amorphous solid: mp 143 °C (with decomposition) (from MeOH); $[\alpha]_D^{26}$ +9.6 (*c* 0.52, $CHCl_3$); IR (KBr) ν_{max} 2950, 2850, 1716, 1715, 1454, 1383, 1181, 640 cm^{-1}; ^1H NMR (300 MHz) δ 4.24 (1H, s, H-15), 2.52 (1H, ddd, J = 16.9, 13.5, 7.0 Hz, H-2α), 2.28 (1H, ddd, J = 16.9, 6.0, 2.8 Hz, H-2β), 2.21 (1H, m, H-11), 2.11 (1H, d, J = 7.9 Hz, H-13), 2.05 (1H, dd, J= 14.3, 4.6 Hz, H-9), 1.99 (1H, m, H-12), 1.98 (1H, m, H-6), 1.81 (1H, m, H-7), 1.77 (1H, m, H-1), 1.71 (1H, m, H-11′), 1.53 (1H, dddd, J = 13.9, 7.8, 4.8, 2.5 Hz, H-6′), 1.38 (1H, ddd, J = 13.4, 13.4, 3.7 Hz, H-1′), 1.34 (3H, s, Me-C_{16}), 1.22 (1H,dd, J= 14.6, 2.1 Hz, H-5), 1.20 (1H, m, H-7′), 1.07 (3H, s, Meβ-C_4), 1.00 (3H, s, Meα-C_4), 0.92 (3H, s, Me-C_{10}); ^{13}C NMR (75 MHz) δ 216.08 (C_3), 206.47 (C_{14}), 56.02 (C_9), 54.44 (C_5), 50.89 (C_{15}), 49.75 (C_8), 47.58 (C_4), 42.77 (C_{13}), 38.04 (C_{16}), 37.55 (C_1), 37.50 (C_{10}), 34.18 (C_{12}), 33.87 (C_2), 28.27 (C_7), 25.57 (C_{18}), 21.49 (C_{19}), 19.36 (C_6), 17.18 (C_{11}), 20.90 (C_{17}), 13.09 (C_{20}); FABMS *m/z* (%) 427 (M^+, 14), 391 (11), 308 (68), 289 (34), 155 (100), 137 (66); HRFABMS *m/z* 427.1148 (calcd for $C_{20}H_{27}IO_2$ 427.1134).

3β-Bromo-19-*nor-ent*-trachylob-4(18)-ene-14,15-dione (23). A solution of NBS (31 mg, 0.17 mmol) in CH_2Cl_2 (0.5 mL) was dropwise added to a solution of compound **32**[9] (40 mg, 0.14 mmol) in CH_2Cl_2 (2.5 mL) at 0 °C. After stirring for 30 min, the reaction mixture was poured into 50 mL of diethyl ether and washed successively with H_2O and brine. The organic phase was dried over $MgSO_4$, and the residue obtained after evaporation of the solvent was purified by chromatography, using hexane−EtOAc (8:2) as eluent, to give the bromodiketone **23** (36 mg, 72%) as a white solid: mp 170.5−172.5 °C (from MeOH); $[\alpha]_D^{29}$ −8 (*c* 0.5, $CHCl_3$); IR (KBr) ν_{max} 2929, 2858, 1752, 1695, 1439, 1203, 1114, 917, 830, 753 cm^{-1}; ^1H NMR (300 MHz) δ 5.13 and 4.74 (1H each, two bs, H_2-18), 4.96 (1H, dd, J = 2.1, 2.1 Hz, H-3), 2.60 (1H, d, J = 7.9 Hz, H-13), 2.50 (1H, dd, J= 7.9, 2.6, 2.6 H-12), 2.4 (1H, m, H-5), 2.26 (1H, dd, J= 10.0, 2.5, H-9), 2.25 and 1.93 (1H each, two m, H_2-11), 1.97 (2H, m, H_2-2), 1.80−1.50 (2H, m, H_2-6), 1.78 (2H, m, H_2-7), 1.72 (1H, m, H-1β), 1.42 (3H, s, Me-C_{16}), 1.35 (1H, ddd, J = 13.4, 3.5, 3.5 Hz, H-1α), 0.64 (3H, s, Me-C_{10}); ^{13}C NMR (75 MHz) δ 207.05 (C_{15}), 206.01 (C_{14}), 148.41 (C_4), 111.09 (C_{18}), 57.90 (C_9), 57.57 (C_3), 55.47 (C_8), 49.33 (C_{16}), 47.12 (C_{13}), 43.62 (C_5), 43.13 (C_{12}), 40.45 (C_{10}), 33.65 (C_1), 31.59 (C_2), 20.87 (C_7), 19.86 (C_{11}), 19.66 (C_6), 12.84 (C_{17}), 11.97 (C_{20}); EIMS *m/z* (%) 394 (M^+, 4), 283(54), 282 (25), 163 (32), 109 (58), 91 (100); HREIMS *m/z* 362.0881 (calcd for $C_{19}H_{23}O_2Br$ 362.0886).

3β-Chloro-19-*nor-ent*-trachylob-4(18)-ene-14,15-dione (24). $Ca(OCl)_2$ (16 mg, 0.11 mmol), H_2O (0.1 mL), and a small piece of solid CO_2 were added to a solution of compound **33**[9] (40 mg, 0.14 mmol) in CH_2Cl_2 (1 mL) at 0 °C. The mixture was stirred for 30 min at the same temperature, poured into 20 mL of H_2O, and extracted with CH_2Cl_2. The combined organic phases were washed with H_2O and brine, dried over $MgSO_4$, and filtered, and the solvent was evaporated under vacuum. Purification by column chromatography, using hexane−EtOAc (8:2) as eluent, afforded chlorodiketone **24** (35 mg, 80%) as a white solid: mp 177−179 °C (from cold MeOH−pentane); $[\alpha]_D^{29}$ −27 (*c* 0.7, $CHCl_3$); IR (KBr) ν_{max} 2929, 2858, 1757, 1705, 1439, 1091, 922, 830, 758, 712 cm^{-1}; ^1H NMR (300 MHz) δ 5.07 and 4.73 (1H each, two bs, H_2-18), 4.72 (1H, m, H-3), 2.60 (1H, d, J = 7.9 Hz, H-13), 2.51 (1H, dd, J = 7.9, 3.0, 3.0 H-12), 2.26 (1H, dd, J = 10.0, 2.5 Hz, H-5), 2.20 and 1.76 (1H each, two m, H_2-11), 2.15 (1H, m, H-9), 1.97 and 1.67 (1H each, two m, H_2-6), 1.92 (2H, m, H_2-2), 1.73 (2H, m, H_2-7), 1.60 (1H, m, H-1β), 1.30 (1H, ddd, J = 13.4, 3.5, 3.5 Hz, H-1α), 1.42 (3H, s, Me-C_{16}), 0.65 (3H, s, Me-C_{10}); ^{13}C NMR (75 MHz) δ 207.05 (C_{14}), 205.98 (C_{15}), 147.87 (C_4), 111.53 (C_{18}), 64.21 (C_3), 57.94 (C_9), 55.49 (C_8), 49.32 (C_{16}), 47.11 (C_{13}), 43.21 (C_5), 43.13 (C_{12}), 40.36 (C_{10}), 32.95 (C_1), 30.87 (C_2), 20.83 (C_7), 19.84 (C_{11}), 19.65 (C_6), 12.83 (C_{17}), 11.59 (C_{20}); EIMS *m/z* (%) 318 (M^+, 36), 282(45), 247 (12), 225 (14), 163 (52), 135 (34), 109 (100), 91 (92); HREIMS *m/z* 318.1387(calcd for $C_{19}H_{23}O_2Cl$ 318.1401).

3β-Hydroxy-19-*nor-ent*-trachylob-4(18)-ene-14,15-dione (25). A solution of compound **32**[9] (35 mg, 0.12 mmol) in CH_2Cl_2 (2 mL) was slowly added to a solution of *m*-CPBA (42 mg, 0.24 mmol) in CH_2Cl_2 (2 mL) at −78 °C. The mixture was stirred while it was allowed to warm to rt. After 10 min, the reaction mixture was quenched with saturated aqueous NH_4Cl solution, poured into H_2O, and extracted with CH_2Cl_2. The combined organic layers were washed sequentially with diluted 5% aqueous $NaHCO_3$ solution and brine and dried over Na_2SO_4. The residue obtained after evaporation of the solvent was filtered through a short pad of silica gel, using hexane−EtOAc (7:3) as eluent, affording a white solid of crude C3−C4 epoxide (36 mg), which was used in the subsequent step without further purification.

A solution of *n*-BuLi in hexane (1.6 M, 0.28 mL, 0.45 mmol) was slowly added to a solution of 2,2,6,6-tetramethylpiperidine (85 μL, 0.49 mmol) in dry benzene (1.5 mL) at 0 °C. After stirring for 10 min at this temperature, a solution of Et_2AlCl in toluene (1.8 M, 0.25 mL, 0.45 mmol) was added dropwise, and the mixture was stirred for 30 min. Then, a solution of the epoxide in dry benzene (0.8 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 1 h and then quenched by the addition of saturated aqueous NH_4Cl solution, poured into cold 1 M aqueous HCl solution, and extracted with EtOAc. The combined organic layers were washed with brine and dried over $MgSO_4$. The residue left after evaporation of the solvent was purified by chromatography, using hexane−EtOAc (6:4) as eluent, to afford the hydroxydiketone **25** (26 mg, 70% overall yield from **32**) as a white solid: mp 229−230 °C (from MeOH); $[\alpha]_D^{29}$ −45 (*c* 0.7, $CHCl_3$); IR (KBr) ν_{max} 3486, 2945, 2852, 1749, 1705, 1443, 1066, 968, 902 cm^{-1}; ^1H NMR (300 MHz) δ 4.99 and 4.66 (1H each, two bs, H_2-18), 4.25 (1H, dd, J = 2.0, 2.0 Hz, H-3), 2.59 (1H, d, J = 7.9 Hz, H-13), 2.48 (1H, dd, J = 7.9, 2.7, 2.7 H-12), 2.25 (1H, ddd, J = 9.9, 9.9, 2.7 Hz, H-11α), 2.17 (1H, m, H-5), 2.17 (1H, m, H-9), 1.92 (1H, ddd, J = 9.9, 4.1, 2.7 Hz, H-11β), 1.65 (2H, m, H_2-7), 1.63 and 1.53 (1H each, two

m, H_2-1), 1.60 (2H, m, H_2-2), 1.51 and 1.24 (1H each, two m, H_2-1), 1.41 (3H, s, Me−C_{16}), 0.64 (3H, s, Me-C_{10}); ^{13}C NMR (75 MHz) δ 207.16 (C_{14}), 206.18 (C_{15}), 150.61 (C_4), 110.35 (C_{18}), 72.80 (C_3), 58.00 (C_9), 55.68 (C_8), 49.31 (C_{16}), 47.16 (C_{13}), 43.49 (C_5), 43.26 (C_{12}), 40.49 (C_{10}), 33.40 (C_1), 29.35 (C_2), 20.96 (C_7), 19.88 (C_{11}), 19.76 (C_6), 12.85 (C_{17}), 11.16 (C_{20}); EIMS *m/z* (%) 300 (M^+, 100), 282(42), 239 (14), 163 (36), 135 (34), 109 (46), 91 (70); HREIMS *m/z* 300.1725 (calcd for $C_{19}H_{24}O_3$ 300.1723).

19-*nor*-*ent*-Trachylob-4(18)-ene-14,15-dione (26). $CrCl_3$ (314 mg, 2 mmol) and $LiAlH_4$ (38 mg, 1 mmol) were suspended in dry THF (2 mL) at rt. When the evolution of hydrogen had ceased, DMF (4 mL) and *i*-PrOH (0.3 mL) were added. After an additional 20 min of stirring, a solution of compound **23** (25 mg, 0.07 mmol) in DMF (1.5 mL) was added. The mixture was stirred for 10 h at rt, and then it was diluted with diethyl ether, washed with 5% aqueous HCl solution and brine, and dried over $MgSO_4$. The solvent was removed under vacuum, and the residue was purified by chromatography, using hexane−EtOAc (8:2), to afford diketone **26** (18 mg, 91%) as a white solid: mp 152−153 °C (from MeOH); $[\alpha]_D^{29}$ −108 (*c* 0.3, $CHCl_3$); IR (KBr) ν_{max} 2929, 2868, 1746, 1695, 1439, 1338, 1239, 968 cm^{-1}; ^{1}H NMR (300 MHz) δ 4.74 and 4.51 (1H each, two bs, H_2-18), 2.60 (1H, d, J = 7.9 Hz, H-13), 2.48 (1H, dd, J = 7.9, 2.3, 2.3 H-12), 2.26 and 1.92 (1H each, two m, H_2-3), 2.24 and 1.92 (1H each, two m, H_2-11), 2.08 (1H, dd, J = 9.8, 6.5 H-9), 1.76−1.63 (2H, m, H_2-7), 1.61−1.51 (2H, m, H_2-6), 1.58 and 1.51 (1H each, two m, H_2-2), 1.57 (1H, m, H-5), 1.50 (1H, m, H-1α), 1.05 (1H, ddd, J = 16.5, 12.3, 2.3 Hz, H-1β), 1.41 (3H, s, Me-C_{16}), 0.65 (3H, s, Me-C_{10}); ^{13}C NMR (75 MHz) δ 207.10 (C_{14}), 206.05 (C_{15}), 149.39 (C_4), 106.98 (C_{18}), 36.36 (C_3), 58.39 (C_9), 55.72 (C_8), 49.21 (C_{16}), 47.14 (C_{13}), 49.78 (C_5), 43.28 (C_{12}), 40.71 (C_{10}), 39.30 (C_1), 22.81 (C_2), 20.98 (C_7), 19.94 (C_{11}), 20.18 (C_6), 12.85 (C_{17}), 11.98 (C_{20} (calcd); EIMS *m/z* (%) 284 (M^+, 100), 269(13), 256 (21), 241 (11), 163 (11), 122 (22), 109 (16), 83(35); HREIMS *m/z* 284.1764 for $C_{19}H_{24}O_2$ 284.1776).

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