Similarities and Differences in the Structure−Activity Relationships of Capsaicin and Resiniferatoxin Analogues

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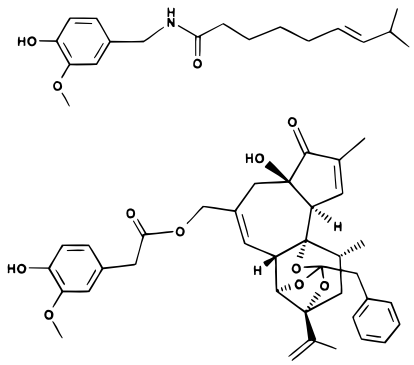
Structure−activity relationships in analogues of the irritant natural product capsaicin have previously been rationalized by subdivision of the molecule into three structural regions (A, B, and C). The hypothesis that resiniferatoxin (RTX), which is a high-potency ligand for the same receptor and which has superficial structural similarities with capsaicin, could be analogously subdivided has been investigated. The effects of making parallel changes in the two structural series have been studied in a cellular functional assay which is predictive of analgesic activity. Parallel structural changes in the two series lead to markedly different consequences on biological activity; the 3- and 4-position aryl substituents (corresponding to the capsaicin 'A-region') which are strictly required for activity in capsaicin analogues are not important in RTX analogues. The homovanillyl C-20 ester group in RTX (corresponding to the capsaicin 'B-region') is more potent than the corresponding amide, in contrast to the capsaicin analogues. Structural variations to the diterpene moiety suggest that the functionalized 5-membered diterpene ring of RTX is an important structural determinant for high potency. Modeling studies indicate that the 3D position of the α-hydroxy ketone moiety in the 5-membered ring is markedly different in the phorbol (inactive) analogues and RTX (active) series. This difference appears to be due to the influence of the strained ortho ester group in RTX, which acts as a local conformational constraint. The reduced activity of an analogue substituted in this region and the inactivity of a simplified analogue in which this unit is entirely removed support this conclusion.

Introduction

Capsaicin and resiniferatoxin (RTX) are irritant natural products which activate the capsaicin (or 'vanilloid') receptor in a subpopulation of primary afferent sensory neurons. These sensory neurons are involved in nociception, and so these agents are targets for the design of a novel class of analgesics. Both ligands cause a novel ion channel in the plasma membrane to become permeable to cations, eliciting a number of biological activities including the excitatory (algesic) as well as the analgesic effects which both agents evoke.

In addition to these biological similarities, a number of groups have drawn attention to structural similarities between the two molecules.[1] Both compounds possess a 3-methoxy-4-hydroxybenzyl group, hydrogen bond donor and acceptor species, and hydrophobic regions. It is generally assumed that the high degree of pharmacological similarity between these two molecules is a consequence of receptor recognition of those structural moieties which are identical or similar in the two compounds. Structure-activity relationships (SAR) for capsaicin agonists have previously been rationalized, by ourselves and others,[2−4] by dividing the capsaicin molecule into three regions: A (aromatic ring), B (amide bond), and C (hydrophobic side chain). We hypothesized that it might be possible to determine if RTX could be so simplistically subdivided and, in doing so, shed light on the nature of the receptor−ligand interactions with both molecules. In particular, elucidation of the structural features of the RTX diterpene unit which are responsible for the extremely high potency of RTX would be invaluable in attempting to increase the potency of simple capsaicinoid analogues. For such an approach to be successful, however, comparable SAR in both series would need to be demonstrated. In order to investigate this hypothesis, parallel modifications to the structure of both ligands was undertaken, and the consequences for biological activity were determined in a well-characterized cellular functional assay (Ca^{2+} uptake assay),[5] which is predictive for analgesic activity *in vivo*.[2]

Chart 1



Chemistry

'α-Region'. C-20 Ester Substitution of Resiniferonol 9,13,14-Orthophenylacetate (ROPA): Synthesis of Substituted Phenylacetic Acids. The FMoc-protected 4-(aminoethoxy)-3-methoxyphenylacetic acid, used in the synthesis of 4-(aminoethoxy)-RTX, **5d**

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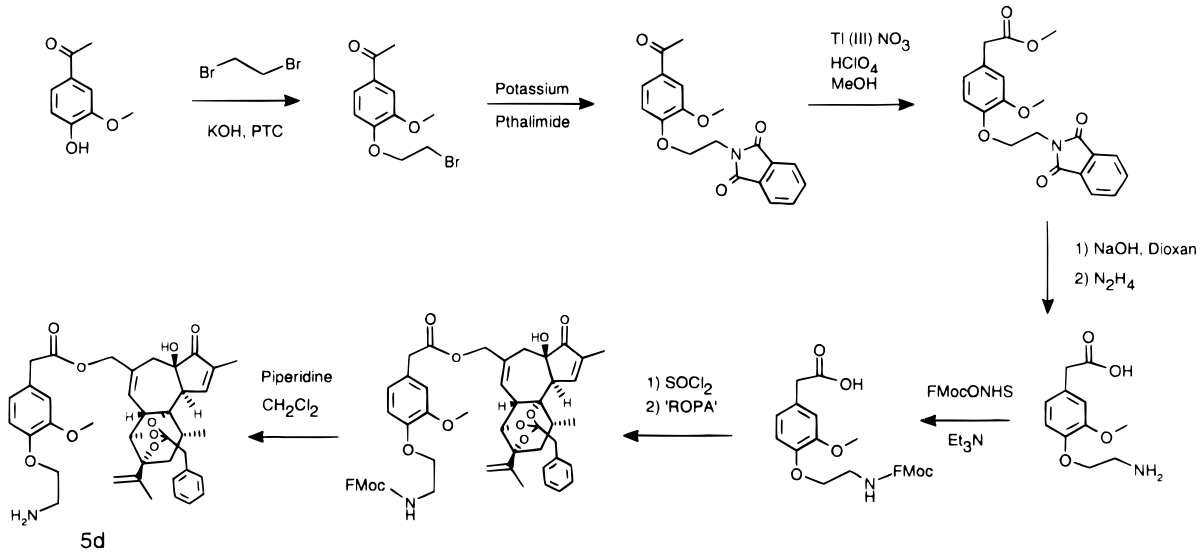
Scheme 1



(Scheme 1), was synthesized from a commercially available acetophenone, having first introduced the 4-phthalimidoethoxy group, using an oxidative thallium(III) rearrangement by the method of McKillop.[6] Saponification followed by hydrazinolysis gave the free amino acid which was FMoc protected using FMoc-succinimide carbonate in the presence of triethylamine.

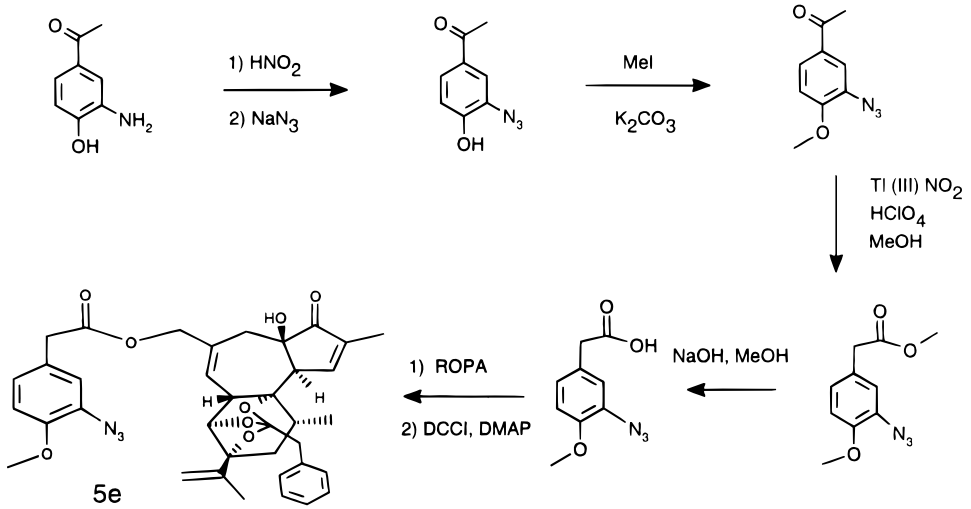
The same thallium rearrangement was also used to access the methyl ester of 3-azido-4-methoxyphenylacetic acid[7] in the synthesis of the photoaffinity label RTX-PAL, **5e** (Scheme 2), after subjecting the commercially available 4-hydroxy-3-aminoacetophenone to diazotization and treatment with sodium azide followed by methylation, all under subdued light. Saponification of the methyl ester gave the substituted phenylacetic acid.

C-20 Esterification. For **4a,b** and **5a**−**d**, the commercially available alcohol ROPA was acylated on the C-20 OH group with the appropriately substituted phenylacetic (or other) acids by conversion of the acid to the acid chloride with thionyl chloride and then condensation with ROPA, in the presence of triethylamine. The esterification yielding **5e**, however, was achieved using DCCI/DMAP. In the case of **5d**, the product of the coupling reaction was deprotected with piperidine in dichloromethane.

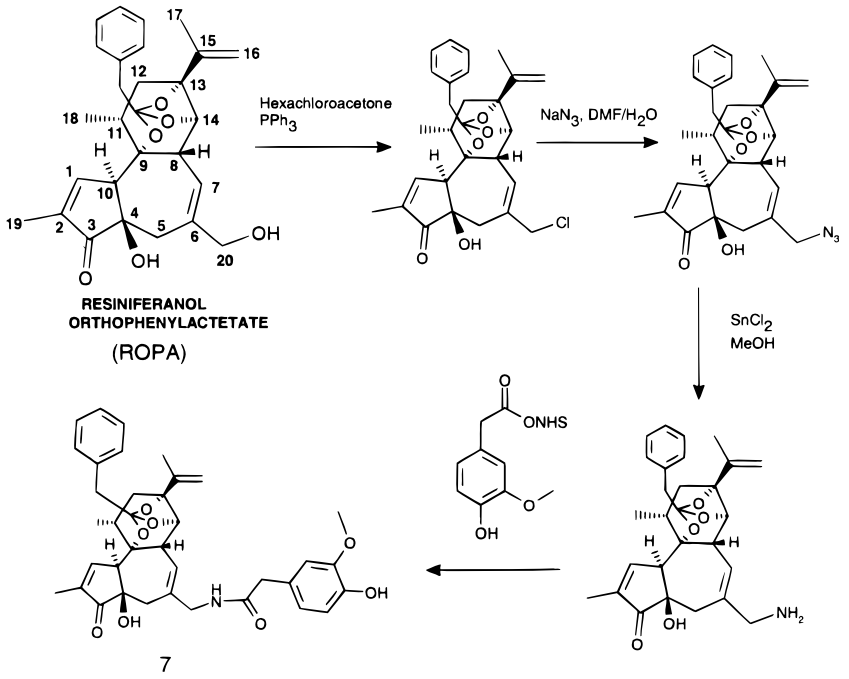
***β*-Region'.** The synthesis of RTX amide (**7**) was achieved by the route shown in Scheme 3. The numbering convention for daphnane diterpenes is shown in the structure of the starting material, resiniferonol 9,-13,14-orthophenylacetate (ROPA). Chlorination of the allylic alcohol ROPA, without rearrangement, was achieved by the method of Magid,[8] using hexachloroacetone and triphenylphosphine. The resulting allyl chloride was treated with sodium azide in DMF to give the allyl azide in quantitative yield. Reduction to the allylamine, resiniferamine 9,13,14-orthophenylacetate, was best achieved using $SnCl_2$ in MeOH.[9] Acylation of the resulting allylamine with homovanillic acid hydroxysuccinimide ester[10] gave RTX amide, **7**. Concurrent with ourselves, the group of Blumberg have developed a similar synthesis of **7**.[11]

***χ*-Region'. C-20 Ester Substitution of Phorbol Esters.** In the case of the phorbol ester derivatives, 12-deoxyphorbol 13-phenylacetate 20-(3-methoxy-4-hydroxyphenylacetate) (**8**),[12] 12,13-diacetylphorbol 20-(3-methoxy-4-hydroxyphenylacetate) (**9a**), and 12,13-didecanoylphorbol 20-(3-methoxy-4-hydroxyphenylacetate) (**9b**), the parent C-20 alcohol was acylated with homovanillic acid using 2-(fluoromethyl)pyridinium tosylate as the coupling reagent. The C-20 formate (presumably

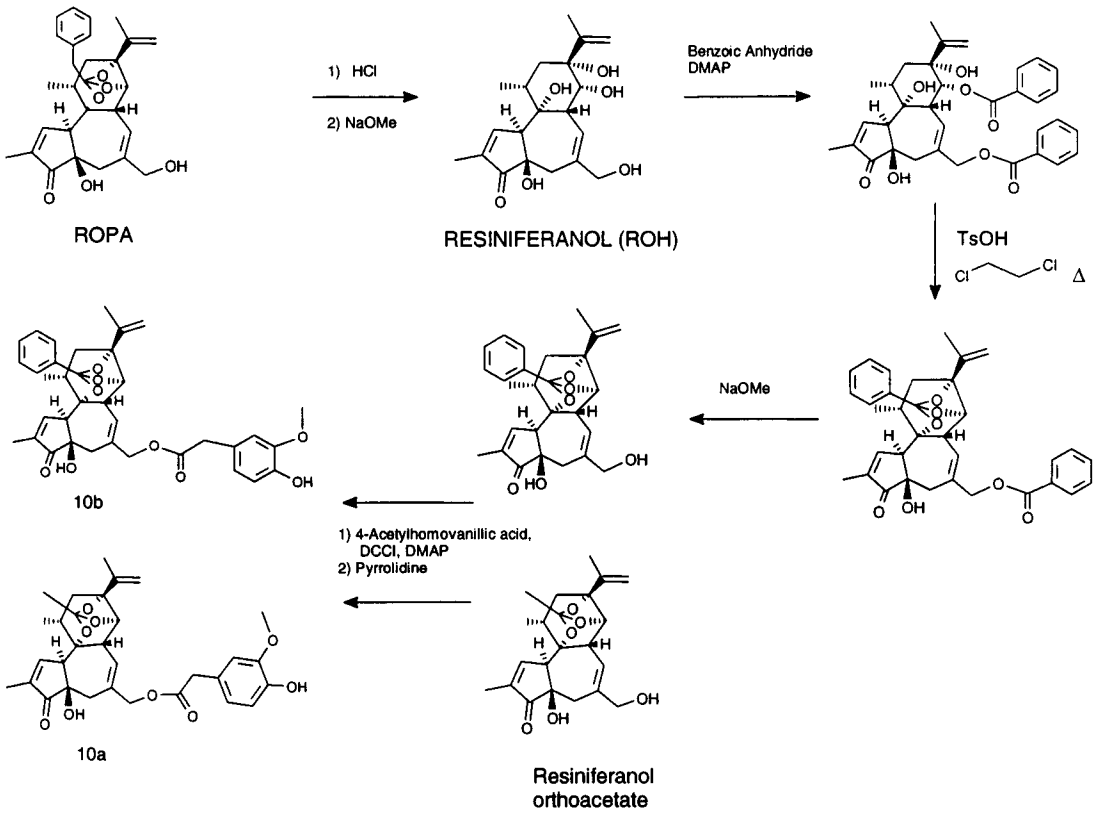
Scheme 2



Scheme 3



Scheme 4



derived from formic acid present in DMF) was formed as a significant side product in all reactions using this reagent but could be separated from product chromatographically.

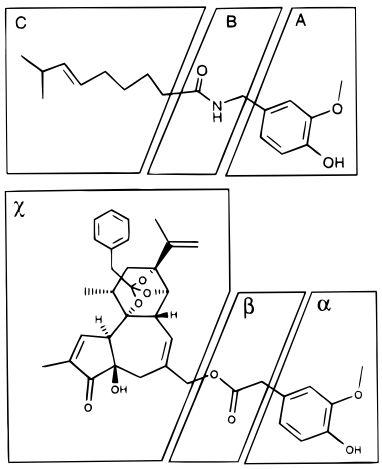
Ortho Ester Substitution. Resiniferonol orthoacetate[13] (Scheme 4) was kindly provided by B. Sorg. The corresponding resiniferonol orthobenzoate was synthesized from the parent diterpene polyol, resiniferonol, by 14,20-dibenzoylation with benzoic anhydride in the presence of DMAP followed by ortho ester cyclization with anhydrous *p*-toluenesulfonic acid in refluxing dichloroethane, which gave the C-20 benzoate 9,13,14-orthobenzoate in quantitative yield. This was C-20 deprotected with sodium methoxide in methanol to give the orthobenzoate C-20 alcohol. Resiniferonol, prepared from ROPA by a modification of the method of Sorg,[13] was obtained in 60% yield, a substantially improved yield compared with that reported by these authors (16.9%) especially since 35% of unreacted ROPA was also recovered from the reaction mixture.

C-20 esterification of resiniferonol orthoacetate and resiniferonol orthobenzoate with acetylhomovanillic acid

Table 1

compd	displacement of [^{3}H]RTX binding (K_i, nM)	relative affinity (RTX)	stimulation of Ca^{2+}uptake (EC_{50}, nM)	relative potency (RTX)
1 (capsaicin)	2000 ± 500		300 ± 40	
2 (RTX)	0.12 ± 0.01	1	1.6 ± 0.1	1
5a	8.3 ± 1.0	0.015	10.22 ± 2.71	0.157
5c	1.0 ± 0.1	0.120	14.89 ± 3.77	0.107
5d	4.3 ± 0.2	0.028	13.04 ± 2.26	0.123
7	10.6 ± 0.9	0.011	122.0 ± 19.0	0.013
8	600 ± 100[16]	0.0002	2450 ± 140	0.0007
10a	3.2 ± 1.1	0.038	13.2 ± 3.8	0.122
10b	0.17 ± 0.02	0.706	7.6 ± 0.6	0.210
11b	4.4 ± 3.4	0.027	57.5 ± 21.2	0.028
ROPA	>10 000	<0.000 01	>20 000	<0.000 08

Chart 2



was effected with DCCI/DMAP coupling, and the products were selectively phenol ester deprotected using pyrrolidine[14] to give orthoacetyl-RTX, **10a**, and orthobenzoyl-RTX, **10b** (Scheme 4).

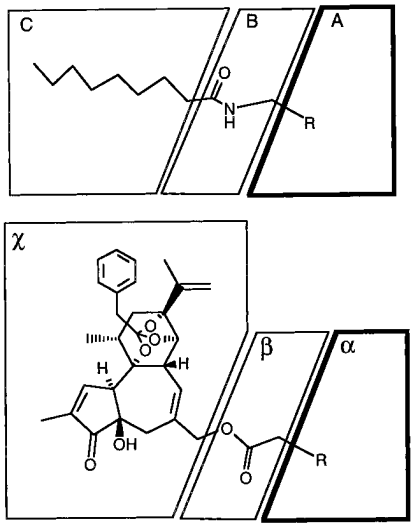
Diterpene Modifications to RTX. The 3-β-OH analogue of RTX (**11b**) was synthesized by direct reduction of RTX with $NaBH_4$ in ethanol. The stereochemistry of the reduction product at C-3 was established by NOE difference NMR spectroscopy. The 4-β-methoxy analogue of RTX (**11a**) was synthesized by C-20 acetylation of ROPA to the acetate (**4a**) followed by methylation with methyl iodide in the presence of silver oxide in DMF. The crude reaction product was transesterified with sodium methoxide in methanol to the parent C-20 alcohol. DCCI/DMAP coupling with acetylhomovanillic acid followed by selective phenolic deacetylation, using pyrrolidine as before, completed the synthesis.

The synthesis by Bloomfield *et al.* of the simplified RTX analogue based on a 2,9,10-trioxatricyclo[4.3.1.0^{3,8}]-decane system (**12**) has been published elsewhere.[15]

Biology. The [^{3}H]RTX binding assay was performed using the published methods.[16] The difference between the affinity of RTX reported in this study and the value we have previously reported[17] is most probably due to the differences in conditions under which the two assays were carried out (37 °C[16] as opposed to room temperature[17] and different techniques for reducing nonspecific binding). All compounds were tested as agonists in the calcium uptake assay.[5]

Biological Activity. In a series of RTX analogues which have been tested in both the Ca^{2+} uptake and [^{3}H]RTX binding assays (Table 1), the biological activity of these compounds was similar, with comparable rank orders of potency being found for the various ligands.

Table 2



A/α-region (R)	caps compd	RTX compd	Ca^{2+} uptake (EC_{50},[2] nM) caps analogues	Ca^{2+} uptake (EC_{50},[2] nM) RTX analogues
H		**4a**		>2000
octyl		**4b**		20 000
(3-H,4-H)Ar	**3a**	**5a**	>100 000	10.22 ± 2.71
(3-OMe,4-H)Ar	**3b**	**5b**	>100 000	7.89 ± 2.51
(3-OMe,4-OH)Ar	**3c**	**2** (RTX)	550 ± 80	1.6 ± 0.1
(3-OMe,4-OMe)Ar	**3d**	**5c**	6410 ± 140	14.89 ± 3.77
(3-OMe,4-$OCH_2CH_2NH_2$)Ar	**3e**	**5d**	410 ± 60[18]	13.04 ± 2.26
(3-N_3,4-OMe)Ar		**5e**		10.64 ± 1.40

It is notable, however, that the potencies in the Ca^{2+} uptake assay are generally about 10-fold lower than binding potencies, presumably due to other processes, *e.g.*, uptake into mitochondria being necessary for detection in this assay. Since the main aim of the present study is to compare the SAR of parallel modifications in both the RTX and capsaicin structural series, subsequent SAR discussion will be restricted to comparisons of Ca^{2+} uptake assay data, since directly comparable data are available for a wide variety of RTX and capsaicin analogues and since correlations between activity in this assay and other assays, including antinociceptive activity *in vivo*, have been established elsewhere.[2]

Results and Discussion

(1) C-20 Ester Substitution: A-Ring SAR. Comparison of the importance of substitution of the aromatic ring in the capsaicin A-region and RTX C-20 ester (α-region) reveals marked differences (Table 2). The C-20 alcohol ROPA and its aliphatic esters, the acetate **4a**[13] and the nonanoate **4b**, are all inactive. The unsubstituted phenylacetyl ester **5a**,[13] however, retains substantial activity, albeit somewhat reduced in comparison with RTX. This contrasts markedly with the case of capsaicin analogues, where the substituents in the 3- and 4-positions of the A-ring are essential for potent agonist activity, exemplified by the inactivity of the unsubstituted analogue **3a**[2]. The phenolic 4-OH group in capsaicin analogues is of particular importance and can only effectively be substituted by the 4-aminoethoxy group, as exemplified by **3e**.[18] This group, presumably, is able to mimic the H-bond donor/acceptor properties

of the phenol which have been proposed to be important for agonist activity.[2] To compare the importance of 3- and 4-position aromatic substitution in the two series, a series of RTX analogues functionalized in these positions was synthesized.

3- and 4-Position Aryl Ring Substitution. In this series of RTX analogues, the structure of the diterpene and linker groups is unchanged with respect to the natural product, whereas in the comparable capsaicin analogues the amide B-region is retained, as in the natural product, but the C-region has been simplified to the bioequivalent octyl group,[3] *i.e.*, RTX analogues have $\beta = CH_2COO$, χ = ROPA, and capsaicin analogues have B = amide (CH_2NHCO) C = octyl (see Table 2).

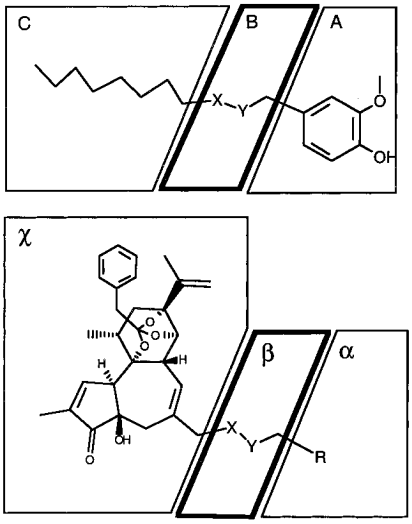
As can be seen from the data in Table 2, the 4-OH group in the RTX analogues is of little importance, since activity is almost completely retained by analogues not containing this substituent, *e.g.*, **5b**. The retention of activity in RTX-PAL, **5e**, enables its use as a photoaffinity label for the capsaicin/vanilloid receptor.[19] In both series the dimethyl ether compound has somewhat lower activity than the parent catechol monomethyl ether. In the case of the RTX analogues, however, the aminoethoxy group in **5d** apparently does not mimic the H-bond donor/acceptor function of the 4-OH group as it appears to in capsaicin analogues.[18]

In conclusion, the C-20 phenylacetyl ester in RTX does appear to be required for high potency, though ring substitution appears much less tightly proscribed than is the case in the capsaicin A-ring. The phenolic OH group which is critical for activity in simple capsaicin analogues has little or no role in RTX analogues. This divergence in SAR is particularly apparent when comparing the coupled pairs **3b** with **5b** (capsaicin analogues) and **5b** with **2** (RTX analogues). Previous studies, using irritant activity as the biological readout,[13,20] also conclude that phenylacetyl esters appear to be most potent, although a more important role for the phenolic OH group is suggested than is indicated in the present study.

(2) C-20 Linker: B-Region SAR. The linker region (β) joining the substituted aromatic α-region to the C-20 position of the diterpene skeleton in RTX could be considered comparable to a 'reverse ester' B-region in a series of capsaicin analogues, *i.e.*, B = CH_2COO. Such a B-region is active in a series of capsaicin analogues (*e.g.*, **6a**[3]) though the ester is somewhat less potent than the corresponding amide (Table 3). By contrast, RTX amide, **7**, is dramatically less potent than the parent ester RTX (**2**). Possible explanations for this large difference between the amide and ester include stabilization of an inactive *trans* C-20 ester conformation by the amide or unfavorable H-bonding or dipolar interactions with the amide NH. The former explanation has been proposed by the group of Blumberg[11] as an explanation for the much lower potency of RTX amide (**7**) than RTX in [^{3}H]RTX binding experiments and for the induction of chemogenic pain and hypothermia *in vivo*.

(3) Diterpene Modification: C-Region SAR. C-Region SAR in capsaicin analogues has been previously published[4] and so is not discussed here in detail. In summary, a hydrophobic group, *e.g.*, an octyl chain or substituted benzyl or phenethyl group, is required for high potency. Optimally, such aralkyl groups are

Table 3



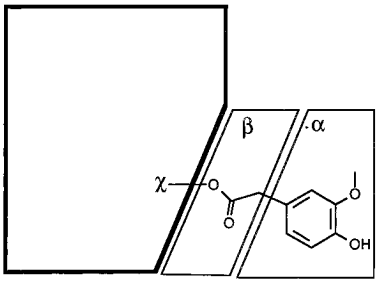
B/β-region	caps compd	RTX compd	Ca^{2+} uptake (EC_{50},[3] nM) caps analogues	RTX analogues
X = O, Y = CO	**6a**	**2** (RTX)	670 ± 110	1.6 ± 0.1
X = NH, Y = CO	**6b**	**7**	300 ± 10	122.0 ± 19.0
X = CO, Y = NH	**3c**		550 ± 80	

substituted in the *para* position by small hydrophobic moieties. The nature of the diterpene group 'χ' has not been thoroughly investigated in previous studies although it is apparent that some derivatives in which the daphnane ortho ester moiety is substituted or exchanged for other diterpenes appear to be much less active than RTX in evoking chemogenic pain[12] or in [^{3}H]-RTX binding experiments.[16] The nature of the ortho ester side chain appears to be important, to some extent, for irritant activity.[13]

Diterpene Replacement. As well as the known 12-deoxyphorbol 20-homovanillic acid ester (**8**),[12] the C-20 homovanillic esters of phorbol 12,13-diacetate and phorbol 12,13-didecanoate (**9a**,**b**, respectively) were prepared. All three compounds showed negligible activity in the Ca^{2+} flux assay (Table 4) and, as such, are even less active than the simple octyl ester capsaicin analogue **6a**[3] (Table 3). Although physicochemical differences (*e.g.*, hydrophobicity) cannot be ruled out as a contributing factor, this result suggests that some highly specific function of the diterpene ortho ester moiety in RTX is responsible for its extremely high potency in this assay.

Superficially, the tricyclic diterpene χ-regions of RTX (**2**) and **8** look very similar, with the same ring junction stereochemistry and identical functionality around the 5- and 7-membered rings, *e.g.*, the $\alpha\beta$-unsaturated α-hydroxy ketone in position C-3/C-4. The only differences occur in the substitution around the 6-membered ring where RTX is oxygenated in a 9α,13α,14α-pattern, while the phorbols and the 12-deoxyphorbol have a 9α,-12β,13α-trioxygenated and 9α,13α-dioxygenated substitution pattern, respectively. In addition, the cyclopropane ring in the phorbols would be expected to constrain the 6-membered ring into a rather different conformation compared with the 13β-isopropenyl group present in RTX. When molecular models of RTX and **8** are overlaid (Figure 1), while the majority of the diterpene skeleton can be superimposed, the region of

Table 4



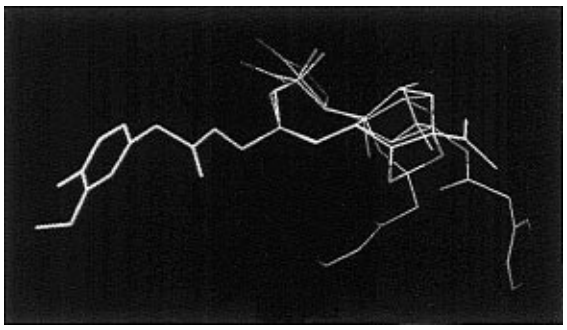
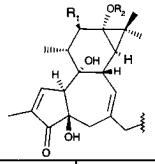
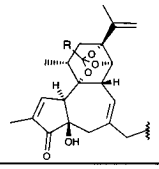


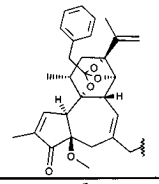
Figure 1. Molecular modeling overlay (best fit of 6- and 7-membered rings) of RTX (**2**) and the 12-deoxyphorbol 13-phenylacetyl ester **8**. The common homovanillyl ester moiety is shown in magenta, the phorbol diterpene skeleton carbon atoms are shown in green, and the RTX diterpene carbon atoms are shown in yellow. All diterpene oxygen atoms are shown in red.



R_1	R_2	Compound No.	Ca^{2+} Uptake EC_{50} nM
H	$COCH_2Ph$	8	2470±140
$OCOCH_3$	$COCH_3$	9a	8510±1990
$OCO(CH_2)_8CH_3$	$CO(CH_2)_8CH_3$	9b	> 10,000



	Compound No.	Ca^{2+} Uptake EC_{50} nM
$R = CH_2Ph$	2 (RTX)	1.6±0.1
$R = CH_3$	10a	13.2±3.8
R = Ph	10b	7.6±0.6



	11a	5.3±0.39
	11b	57.5±21.2
racemate	12	9150±1360

conformational space accessible to the benzyl moiety on the 12-phenylacetate side chain in **8** is clearly much greater and tends to assume a rather equatorial arrangement (approximately coplanar) with respect to the 6-membered ring, which exists in a chair conformation. By contrast, in RTX, the benzyl moiety of the orthophenylacetate side chain is much more constrained and held approximately perpendicular with respect to the 6-membered ring which, in this case, is held in a boat conformation. Another interesting feature of phorbol/RTX analogue overlays, exemplified by Figure 1, is that the orientation of the C-3 ketone carbonyl group with respect to the superimposable 7-membered rings is markedly different in the two structural series. This effect, presumably, is a consequence of the local conformational constraints imposed by the cyclic ortho ester in the daphnane system on the 6-membered ring, being transferred to the rings to which it is fused. Analogues of RTX were therefore synthesized in which the nature of the ortho ester side chain was varied in an attempt to identify the most important feature of the ortho ester in RTX for activity: (1) presentation of a precisely oriented hydrophobic group or (2) a local conformational constraint with the potential of influencing other important receptor interactions.

Ortho Ester Side Chain Modifications. To investigate the possibility of a precisely located hydrophobic binding site, which recognizes the ortho ester side chain, analogues of RTX were synthesized in which the orthophenylacetyl group (benzyl side chain) was replaced by an orthoacetyl and orthobenzoyl group (methyl and phenyl side chains, respectively). While the orthobenzoyl compound **10b** retains high potency, comparable to RTX (in both the Ca^{2+} uptake and binding assays, Table 4; see also Table 1), there is a significant loss of potency in the case of the orthoacetate **10a**, which is particularly apparent from the binding data (Table 1). This compound does, however, still remain much more potent than any of the phorbol analogues or the simple octyl ester capsaicin analogue **6a**.[3] These data suggest that the more important effect of the ortho ester is to act as a local conformational constraint, though there does appear to be a relatively small additive requirement for a hydrophobic or larger group substituting the central ortho ester carbon atom. A further possibility, that direct receptor recognition of the ortho ester oxygen atoms underlies the extremely high potency of these ligands, seems unlikely in view of the high potency of the orthobenzoate **10b**, in which these oxygen atoms would be occluded from contact with the receptor by the bulky phenyl ring, and the lower potency of the orthoacetate **10a**, in which such an interaction should be more favorable, on steric grounds.

Daphnane Skeleton Modifications. The above argument suggests that the ortho ester group may exert a remote conformational effect on the daphnane ortho

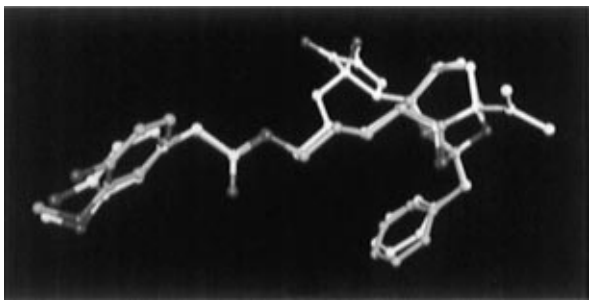


Figure 2. Molecular modeling overlay (best fit of 6-membered ring and ortho ester) of RTX (**2**; shown in magenta) and the simplified RTX analogue **12** (shown in blue).

ester skeleton which places key structural features in the correct orientation for receptor recognition. From the modeling work, a likely candidate for such an important structural feature is the $\alpha\beta$-unsaturated α-hydroxy ketone moiety in positions C-3 and C-4 of the diterpene. In order to test this hypothesis, analogues of RTX were prepared in which both the carbonyl and hydroxy groups were modified. While methylation of the 4-β-OH group, in compound **11a**, has a negligible effect on activity, reduction of the 3-keto carbonyl group to the 3-β,4-β-diol (**11b**) results in a substantial loss of potency (Table 4).

A simplified analogue (**12**), described by Bloomfield *et al.*,[15] which possesses a cyclohexane phenylacetyl ortho ester linked to a homovanillic ester *via* an allylic alcohol, following the backbone of RTX, has very low potency, being less active than the simple octyl ester capsaicin analogue **6a**[3]. Since **12** and RTX can easily be overlaid, as shown in Figure 2, the correct orientation of the substituted phenylacetate ortho ester *per se* does not seem to be sufficient for high potency. It is doubtful that the increased conformational freedom which results from the formal removal, in **12**, of the fused 7-membered diterpene ring present in RTX could alone result in such a dramatic loss in activity.

Overall Conclusions. The loss of potency on reduction of the 3-keto group, taken together with the different orientation of this group in RTX (active) and phorbol (inactive) analogues with otherwise comparable diterpene functionality, suggests an important role for the functional groups substituting the 5-membered ring in RTX, especially the 3-keto group. The inactivity of the simplified RTX analogue described by Bloomfield *et al.*,[18] which contains the phenylacetyl ortho ester moiety but not the fused 7- or 5-membered rings, is consistent with this suggestion. Since the 5-membered ring substituents in RTX could make potential hydrogen-bonding interactions with the receptor, perhaps these groups, and not the linker group β, should be viewed as a counterpart of the capsaicin B-region, especially since the B-region/β-region SAR is so different between the two series.

Attempts to mimic the 3D position and orientation of the 3-keto group in the RTX diterpene moiety in a simple capsaicin analogue would be an important step in testing this hypothesis and could lead to the design of simple capsaicin analogues with higher potencies than have so far been achieved.

Experimental Section

General Information. Routine NMR spectra were recorded using a Varian Gemini 200 machine. High-field spectra were recorded using Varian VX400 400 MHz (University College London Chemistry Department) and Bruker AM500 500 MHz (Sandoz, Basel) instruments. All spectra were recorded using tetramethylsilane (TMS) as an internal standard, and chemical shifts are reported in parts per million (δ) downfield from TMS. Coupling constants are reported in hertz. Mass spectra were recorded by the Mass Spectrometry Department of University College London, using a VG 7070F/H spectrometer, and FAB spectra were recorded in Sandoz, Basel, using a VG 70-SE spectrometer. Accurate mass determinations were made by M. Cocksedge and Dr. D. Carter, London School of Pharmacy, using a VG ZAB SE mass spectrometer and FAB ionization.

TLC was performed using Merck Kiesel gel 60 F_{254} silica plates, and components were visualized using UV light and iodine vapor. HPLC was performed using a Waters 600 system (μ-Bondapak C-18 column (RP_{18}), using gradients or isocratic solvent systems of compositions stated in the text). Compounds were purified by flash column chromatography[21] using Merck Kiesel gel 60 (230−400 mesh) or preparative HPLC using a Waters Delta Prep 3000 preparative chromatography system equipped with a Dynamax 300A C18 12 μm particle size column (83−243-C), dimensions 41.4 × 250 mm. Solvents were HLPC grade and used without further purification. Solvents were dried according to the standard procedures.[22] Test compounds were homogeneous by TLC or HPLC unless otherwise stated. Chemical yields were not optimized.

Resiniferatoxin (RTX) and resiniferonol 9,13,14-orthophenylacetate (ROPA) were obtained from CCR Inc., KS, and were pure by HPLC. In both cases, NMR and MS spectra, including HRMS data, confirmed the identity of the compound. Resiniferonol 9,13,14-orthoacetate[13] was kindly provided by B. Sorg. 12-Deoxyphorbol 13-phenylacetate, phorbol 12,13-diacetate, and phorbol 12,13-didecanoate were obtained from LC Services.

Safety Information. All the diterpene final compounds described in this article should be assumed to be extremely irritant compounds. All diterpene intermediates and final products should, in addition, be treated as potential tumor promotors, especially esters of phorbol. Great care should be taken to avoid exposure. When transferring a known weight of compound is necessary, solutions in a known volume of dry dichloromethane or acetone should be made and the desired amount of solution aliquoted into the reaction vessel or vial for testing, as required. Solvent should then be removed by rotory evaporation *in vacuo* or by passing a stream of dry nitrogen over the sample until a constant weight of diterpene, as a glassy resin, remains as a film inside the vessel.

RTX Analogues: 3-Azido-4-hydroxyacetophenone. 3-Amino-4-hydroxyacetophenone (3.1 g, 20 mmol) was dissolved in water (8 mL), and concentrated HCl solution (4.5 mL) was added. The cream suspension was stirred at 0 °C while a solution of sodium nitrite (1.45 g, 21 mmol) in water (5 mL) was added, dropwise. After stirring for 15 min, the orange solution was filtered and stirred at 0 °C during the addition of a solution of sodium azide (1.3 g, 20 mmol) in water (5 mL). The frothing solution was stirred until N_2 evolution ceased (30 min), and the yellow precipitate was then collected by filtration under subdued light and dried *in vacuo* to give a yellow solid, yield 2.3 g (64%): TLC (silica gel, CH_2Cl_2/MeOH, 20:1) $R_f = 0.44$; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.58 (3H, s, $ArCH_3$), 6.25 (1H, br s, ArOH), 6.98 (1H, d, $J = 8$ Hz, ArH_5), 7.69 (1H, d of d, $J = 8$ Hz, $J' = 2$ Hz, ArH_6), 7.76 (1H, d, $J' = 2$ Hz, ArH_2).

3-Azido-4-methoxyacetophenone. A solution of 3-azido-4-hydroxyacetophenone (2.2 g, 12.4 mmol) in acetone (60 mL) and solid K_2CO_3 (1.71 g, 12.4 mmol) was stirred under a N_2 atmosphere. Methyl iodide (7.1 g, 50 mmol) was added and the mixture refluxed, under subdued light, for 2 h. The crude product was purified by flash column chromatography (silica gel, cyclohexane/EtOAc, 2:1) to give a beige solid, yield 1.8 g (76%): TLC (silica gel, cyclohexane/EtOAc, 1:1) $R_f = 0.40$; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.53 (3H, s, $ArCH_3$), 3.92 (3H, s, $ArOCH_3$), 5.90 (1H, d, $J = 8$ Hz, ArH_5), 7.60 (1H, d, $J' = 2$ Hz, ArH_2), 7.72 (1H, d of d, $J = 8$ Hz, $J' = 2$ Hz, ArH_6).

Methyl 3-Azido-4-methoxyphenylacetate.[7] Thallium(III) nitrate (2.75 g, 6.3 mmol) was dissolved in a 17% solution

of perchloric acid (70% aqueous) in methanol (18.6 mL) and stirred at room temperature. A solution of 3-azido-4-methoxyacetophenone (1.2 g, 6.3 mmol) in the same methanolic perchloric acid solution (10 mL) was added and the reaction mixture stirred, under subdued light, for 18 h. After this time significant starting material (~30%) remained by TLC, and so additional thallium nitrate (1 g, 2.3 mmol) was added and the reaction mixture stirred for a further 1 h, after which time no starting material remained by TLC. The reaction mixture was poured into water (1 L) and extracted with EtOAc, and the extract was dried over Na_2SO_4. The crude product was purified by flash column chromatography (silica gel, cyclohexane/EtOAc, 2:1) to give a pale yellow oil, yield 0.7 g (50%): TLC (silica gel, cyclohexane/EtOAc, 1:1) $R_f = 0.51$; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.51 (2H, s, $ArCH_2CO$), 3.66 (3H, s, $ArCH_2COOCH_3$), 3.82 (3H, s, $ArOCH_3$), 6.81 (1H, d, $J = 8$ Hz, ArH_5), 6.90 (1H, d, $J' = 2$ Hz, ArH_2), 6.96 (1H, d of d, $J = 8$ Hz, $J' = 2$ Hz, ArH_6).

3-Azido-4-methoxyphenylacetic Acid. Methyl 3-azido-4-methoxyphenylacetate (0.65 g, 2.2 mmol) was dissolved in dioxane (25 mL), 5 N NaOH (6 mL, 29 mmol) was added, and the reaction mixture was stirred for 4 h at room temperature under subdued light. The dioxane was then removed *in vacuo*, and the remaining aqueous solution was carefully acidified with HCl (concentrated) until a beige solid precipitated. The mixture was extracted with EtOAc, and dried over Na_2SO_4, and evaporated to give a yellow gum. The crude product was purified by flash column chromatography (silica gel, cyclohexane/EtOAc, 1:1) to give a pale yellow glass, yield 0.42 g (69%): ^{1}H NMR (CD_3OD, 200 MHz) δ 3.39 (2H, s, $ArCH_2CO$), 3.83 (3H, s, $ArOCH_3$), 6.92 (1H, d, $J = 8$ Hz, ArH_5), 6.96 (1H, d, $J' = 2$ Hz, ArH_2), 7.06 (1H, d of d, $J = 8$ Hz, $J' = 2$ Hz, ArH_6).

9,13,14-Orthophenylacetylresiniferonyl 20-(3-Azido-4-methoxyphenylacetate) (5e). Resiniferonol 9,13,14-orthophenylacetate (20 mg, 0.043 mmol) was dissolved in dry CH_2Cl_2 (2 mL), (dimethylamino)pyridine (0.57 mg, 0.0047 mmol) was added, and the solution was stirred at room temperature under a N_2 atmosphere in the dark. A solution of dicyclohexylcarbodiimide (DCCI; 9.7 mg, 0.047 mmol) in CH_2Cl_2 (0.5 mL) and then a solution of 3-azido-4-methoxyphenylacetic acid (9.8 mg, 0.047 mmol) in CH_2Cl_2 (0.5 mL) was added, and the reaction mixture was stirred for 1 h. The solution was washed with 1 M $NaHCO_3$ and then NaCl (saturated) and dried over $MgSO_4$. After evaporation *in vacuo*, the crude product was purified by preparative reversed-phase HPLC (isocratic MeOH (83%)/H_2O (17%), no UV monitor). The pure fractions identified by analytical HPLC were pooled and evaporated under subdued light to give a colorless glass, yield 27.6 mg (98%): TLC (silica gel, cyclohexane/EtOAc, 1:1) $R_f =$ 0.48 (colorless, darkens in light); HPLC (isocratic MeOH (83%)/H_2O (17%)) $t_R = 5.8$ min, 100% pure; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.96 (3H, d, CH_3 H$_3$-18), 1.53 (3H, s, CH_3 H$_3$-17), 1.83 (3H, br d, CH_3 H$_3$-19), 2.02−2.24 (3H, m, H-5α, H-12α,β), 2.47 (1H, AB, H-5β), 2.56 (1H, m, H-11), 3.09 (2H, br s, H-10, H-8), 3.22 (2H, s, ortho ester CH_2Ph), 3.57 (2H, s, phenylacetyl ester $ArCH_2CO$), 3.84 (3H, s, $ArOCH_3$), 4.20 (1H, d, H-14), 4.56 (2H, AB, H$_2$-20), 4.71 (2H, s, H$_2$-16), 5.87 (1H, m, H-7), 6.83 (1H, d, $J = 8$ Hz, ArH_5), 6.94 (1H, d, $J' = 2$ Hz, ArH_2), 7.03 (1H, d of d, $J = 8$ Hz, $J' = 2$ Hz, ArH_6), 7.25−7.50 (6H, m, phenylacetyl ortho ester 5ArH, H-1); FAB-MS $(M + 1)^+$ 654 (92). Accurate mass (FAB MH^+): calcd for $C_{37}H_{40}N_3O_8$, 654.2315; found, 654.2310.

9,13,14-Orthophenylacetylresiniferonyl 20-Chloride.[11] Resiniferonol 9,13,14-orthophenylacetate (100 mg, 0.22 mmol) was dissolved in hexachloroacetone (700 μL, 4.8 mmol) and stirred on an ice bath. Triphenylphosphine (62.1 mg, 0.24 mmol) in CH_2Cl_2 (200 μL) was slowly added. After stirring for 15 min, no starting material remained by TLC. The solvent was evaporated *in vacuo*, and the crude product was purified by preparative reversed-phase HPLC (isocratic MeOH (83%)/H_2O (17%)). The pure fractions were pooled and evaporated to give a colorless glass, yield 90 mg (87%): TLC (silica gel, cyclohexane/EtOAc, 1:1) $R_f = 0.59$; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.96 (3H, d, CH_3 H$_3$-18), 1.52 (3H, s, CH_3 H$_3$-17), 1.58 (1H, d, H-12 α), 1.83 (3H, br d, CH_3 H$_3$-19), 2.15 (1H, m, H-12β), 2.32 (1H, AB, H-5α), 2.56 (1H, m, H-11), 2.70 (1H, AB, H-5β), 3.15 (2H, br s, H-10, H-8), 3.21 (2H, s, ortho ester CH_2Ph), 4.12 (2H, AB, CH_2Cl H$_2$-20), 4.24 (1H, d, H-14), 4.71 (2H, s, H$_2$-16), 6.01 (1H, m, H-7), 7.20−7.40 (5H, m, phenylacetyl ortho ester ArH), 7.46 (1H, m, H-1); FAB-MS $(M + 1)^+$ 483 (100).

9,13,14-Orthophenylacetylresiniferonyl 20-Azide.[11] 9,13,14-Orthophenylacetylresiniferonyl 20-chloride (70 mg, 0.15 mmol) was dissolved in DMF and stirred at room temperature. A solution of sodium azide (10.7 mg, 0.17 mmol) in 10:2 DMF/H_2O was added and the mixture stirred for 90 min, after which time reaction was complete by TLC. The solvent was removed *in vacuo* and 50/50% diethyl ether/CH_2Cl_2 (10 mL) added to the resulting resin. The insoluble salts were removed by filtration, and the organic solution was concentrated *in vacuo* to leave a colorless glass (70 mg, 100%): pure by TLC (silica gel, cyclohexane/EtOAc, 1:1) $R_f = 0.52$; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.95 (3H, d, CH_3 H$_3$-18), 1.52 (3H, s, CH_3 H$_3$-17), 1.65 (1H, d, H-12α), 1.82 (3H, br d, CH_3 H$_3$-19), 2.12 (1H, t, H-12β), 2.18 (1H, AB, H-5α), 2.50 (1H, m, H-11), 2.65 (1H, AB, H-5β), 3.15 (2H, br s, H-10, H-8), 3.22 (2H, s, ortho ester CH_2Ph), 3.79 (2H, br s, CH_2N_3 H$_2$-20), 4.26 (1H, d, H-14), 4.63 (2H, s, H$_2$-16), 5.89 (1H, br m, H-7), 7.20−7.40 (5H, m, phenylacetyl ortho ester ArH), 7.46 (1H, br s, H-1); FAB-MS $(M + 1)^+$ 490 (100).

9,13,14-Orthophenylacetylresiniferonylamine.[11] Tin(II) chloride (67 mg, 0.36 mmol) was dissolved in anhydrous methanol (1 mL) and stirred at room temperature under a N_2 atmosphere. 9,13,14-Orthophenylacetylresiniferonyl 20-azide (42 mg, 0.086 mmol), in solution in anhydrous methanol (0.5 mL), was slowly added. The reaction mixture was stirred for 8 h, after which time no starting material remained by TLC. The solvent was removed *in vacuo*, and the residue was redissolved in 1 M NaOH (2 mL) and then extracted with CH_2Cl_2 (10 mL). The organic solution was dried over $MgSO_4$ and then evaporated to give a colorless glass, yield 30 mg (75%) which was >90% pure by TLC and used in the next step without purification: TLC (silica gel, CH_2Cl_2/MeOH/HOAc, 90:9:1) $R_f = 0.08$ (stains strongly with ninhydrin and fluorescamine); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.95 (3H, d, CH_3 H$_3$-18), 1.52 (3H, s, CH_3 H$_3$-17), 1.55 (1H, d, H-12α), 1.86 (3H, br d, CH_3 H$_3$-19), 2.10−2.25 (2H, m, H-12b, H-5α), 2.55−2.65 (2H, m, H-11, H-5β), 3.11 (2H, br s, H-10, H-8), 3.21 (2H, s, ortho ester CH_2Ph), 3.28 (2H, d, CH_2NH_2 H$_2$-20) 4.25 (1H, d, H-14), 4.72 (2H, s, H$_2$-16), 5.76 (1H, br s, H-7), 7.25−7.40 (5H, m, phenylacetyl ortho ester ArH), 7.46 (1H, br s, H-1); FAB-MS $(M + 1)^+$ 490 (100).

***N*-(9,13,14-Orthophenylacetylresiniferonyl)-4-hydroxy-3-methoxyphenylacetamide**[11] **(7).** 9,13,14-Orthophenylacetylresiniferonylamine (22 mg, 0.048 mmol) was dissolved in dry CH_2Cl_2 (2 mL) and stirred on ice under a N_2 atmosphere. Homovanillic acid *N*-hydroxysuccinimide ester[8] (12.7 mg, 0.048 mmol), as a solution in CH_2Cl_2 (0.5 mL), was added and the reaction mixture stirred for 18 h. The reaction mixture was washed with water and then saturated NaCl and dried over $MgSO_4$. The solvent was removed *in vacuo* leaving a colorless glass which was purified by preparative reversed-phase HPLC (isocratic MeOH (70%)/H_2O (30%)). The pure fractions identified by analytical HPLC were pooled and evaporated to give a colorless glass, yield 8 mg (27%): TLC (silica gel, CH_2Cl_2/MeOH, 25:1) $R_f = 0.19$; HPLC (MeOH/H_2O gradient 60−90% MeOH) $t_R = 10.6$ min, 100% pure; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.94 (3H, d, CH_3 H$_3$-18), 1.20 (1H, d, H-12 α), 1.54 (3H, s, CH_3 H$_3$-17), 1.81 (3H, br d, CH_3 H$_3$-19), 2.02 (1H, AB, H-5α), 2.12 (1H, d, H-12β), 2.40 (1H, AB, H-5β), 2.54 (1H, m, H-11), 3.01 (2H, br s, H-10, H-8), 3.21 (2H, s, ortho ester CH_2Ph), 3.56 (2H, s, $ArCH_2CONH$), 3.66 (2H, m, $CONHCH_2$ H$_2$-20), 3.82 (1H, br s, ArO*H*), 3.86 (3H, s, $ArOCH_3$), 4.16 (1H, d, H-14), 4.71 (2H, s, H$_2$-16), 5.52−5.60 (2H, br m, ArOH, CH_2NHCO), 5.62 (1H, s, H-7), 6.74−6.90 (3H, m, vanillyl ArH), 7.25−7.50 (6H, m, phenylacetyl ortho ester 5ArH, H-1). FAB-MS $(M + 1)^+$ 628 (100). Accurate mass (FAB MH^+): calcd for $C_{37}H_{42}NO_8$, 628.2910; found, 628.2914.

4-(Bromoethoxy)-3-methoxyacetophenone. Acetovanillone (5 g, 30 mmol), 1,2-dibromoethane (89 mL, 194 g, 1.04 mol), 40% KOH (21 mL, 150 mmol), and 40% tetrabutylammonium hydroxide (20 mmol) were combined and heated to 55 °C with stirring. No acetovanillone remained by TLC after

3 h. The cooled reaction mixture was diluted with CH_2Cl_2 (200 mL), extracted with water (2 × 100 mL), washed with saturated NaCl, and then dried over Na_2SO_4. After removal of the solvents *in vacuo*, a yellow crystalline solid remained, yield 7.89 g (95%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.60 (3H, s, ArCOCH_3), 3.66 (2H, m, CH_2CH_2Br), 3.96 (3H, s, ArOCH_3), 4.42 (2H, m, ArOCH_2CH_2Br), 6.93 (1H, d, J = 8 Hz, ArH_5), 7.50−7.70 (2H, m, ArH_2, ArH_6).

4-(Phthalimidoethoxy)-3-methoxyacetophenone. 4-(Bromoethoxy)-3-methoxyacetophenone (7.5 g, 28 mmol) was added to dry DMF (50 mL) and stirred at 55 °C until solubilized. Potassium phthalimide (6.5 g, 35 mmol) was added and the mixture stirred at 55 °C for 2 h, after which time no starting material remained by TLC. The solvent was removed *in vacuo*, redissolved in EtOAc, washed with water and saturated NaCl, and dried over $MgSO_4$. The product, on evaporation of the solvent, was recrystallized from ethanol to give cream needles, yield 6.7 g (72%): TLC (silica gel, cyclohexane/EtOAc, 1:2) R_f = 0.50; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.50 (3H, s, ArCOCH_3), 3.70 (3H, s, ArOCH_3), 4.02 (2H, t, ArOCH$_2CH_2$N[phthal]), 4.35 (2H, t, ArOCH_2CH$_2$N[phthal]), 7.10 (1H, d, J = 8 Hz, ArH_5), 7.45−7.70 (2H, m, ArH_2, ArH_6).

Methyl 4-(Phthalimidoethoxy)-3-methoxyphenylacetate. Thallium(III) nitrate (2.62 g, 6.0 mmol) was dissolved in a 17% solution of perchloric acid (70% aqueous) in methanol (17.7 mL) and stirred at room temperature. A solution of 4-(phthalimidoethoxy)-3-methoxyacetophenone (2 g, 6.0 mmol) in the same methanolic perchloric acid solution (10 mL) was added and the reaction mixture stirred for 18 h, after which time no starting material remained by TLC. The reaction mixture was poured into water (1 L) and extracted with EtOAc, and the extract was dried over Na_2SO_4. The crude product was purified by flash column chromatography (silica gel, cyclohexane/EtOAc, 2:1) to give a white solid, yield 1.5 g (69%): TLC (silica gel, cyclohexane/EtOAc, 1:1) R_f = 0.26; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.52 (2H, s, ArCH_2COCH$_3$), 3.70 (3H, s, ArCH$_2$COCH_3), 3.80 (3H, s, ArOCH_3), 4.10−4.30 (4H, m, ArOCH$_2CH_2$N[phthal], ArOCH_2CH$_2$N[phthal]), 6.70−6.90 (3H, m, vanillyl ArH), 7.60−7.90 (4H, m, phthalimide ArH).

4-(Phthalimidoethoxy)-3-methoxyphenylacetic Acid. Methyl 4-(phthalimidoethoxy)-3-methoxyphenylacetate (3.0 g, 8.2 mmol) was dissolved in dioxane (65 mL), and 5 M NaOH (16.3 mL, 82 mmol) was added. The reaction mixture was stirred at room temperature for 18 h, after which time no starting material reamained by TLC. The dioxane was removed *in vacuo* leaving an aqueous solution which was acidified with HCl (concentrated) causing precipitation of a white solid which was extracted with EtOAc. The extract was washed with NaCl (saturated) and dried over Na_2SO_4. Evaporation of the solvent gave a white crystalline solid, yield 2.75 g (100%): TLC (silica gel, cyclohexane/EtOAc, 1:1) R_f = 0.02; ^{1}H NMR (CD_3OD, 200 MHz) δ 3.55 (2H, s, ArCH_2CO_2H), 3.72 (2H, m, ArOCH$_2CH_2$N[phthal]), 3.78 (3H, s, ArOCH_3), 4.15 (2H, t, ArOCH_2CH$_2$N[phthal]), 6.80−6.95 (3H, m, vanillyl ArH), 7.30−8.00 (4H, m, phthalimide ArH).

4-(Aminoethoxy)-3-methoxyphenylacetic Acid. 4-(Phthalimidoethoxy)-3-methoxyphenylacetic acid (1.5 g, 4.4 mmol) was dissolved in ethanol (7.5 mL), hydrazine monohydrate (1.74 mL, 35.8 mmol) was added, and the mixture was refluxed for 2 h. The cooled reaction mixture was filtered and the filtrate evaporated *in vacuo* to give a gum which was redissolved in MeOH (20 mL). The solution was diluted with EtOAc (100 mL) which caused an oil to separate. The solvent was decanted from the oil, the residue was washed with hexane, and solvent residues were evaporated *in vacuo*, leaving a colorless gum, yield 0.8 g (80%): TLC (silica gel, CH_2Cl_2/MeOH/HOAc, 120:90:5) R_f = 0.43 (purple stain with ninhydrin); ^{1}H NMR (CD_3OD, 200 MHz) δ 3.15 (2H, t, ArOCH$_2CH_2NH_2$), 3.42 (2H, s, ArCH_2CO), 3.84 (3H, s, ArOCH_3), 4.10 (2H, br t, ArOCH_2CH$_2$NH$_2$), 6.80−7.00 (3H, m, vanillyl ArH).

4-[[(Fluorenylmethyloxycarbonyl)amino]ethoxy]-3-methoxyphenylacetic Acid. 4-(Aminoethoxy)-3-methoxyphenylacetic acid (0.4 g, 1.78 mmol) was dissolved in water (3 mL), and triethylamine (0.27 mL, 1.96 mmol) was added (final pH ∼ 10). A solution of FMoc-succinimide carbonate (0.65 g, 1.93 mmol) in CH_3CN (3 mL) was added and the reaction mixture stirred at room temperature for 2 h. The solvent was removed *in vacuo*, and the residue was redissolved in EtOAc, washed with an aqueous solution of $KHSO_4$ (1%) and NaCl (saturated), and then dried over $MgSO_4$. The solvent was removed *in vacuo*, and the crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH, 10:1). The pure fractions were evaporated to give a cream solid, yield 320 mg (40%): TLC (silica gel, CH_2Cl_2/MeOH, 10:1) R_f = 0.23; ^{1}H NMR (CD_3OD, 200 MHz) δ 3.35 (2H, m, fluorenylCH_2O), 3.50 (2H, s, ArCH_2CO), 3.75 (3H, s, ArOCH_3), 3.90 (1H, m, fluorenyl 5-ring CH), 4.10−4.50 (4H, m, ArOCH_2CH_2NH), 6.82−6.89 (3H, m, vanillyl ArH), 7.19−7.79 (8H, m, fluorenyl ArH).

9,13,14-Orthophenylacetylresiniferonyl 20-[4-[[(Fluorenylmethyloxycarbonyl)amino]ethoxy]-3-methoxyphenylacetate]. 4-[[(Fluorenylmethyloxycarbonyl)amino]ethoxy]-3-methoxyphenylacetic acid (50 mg, 0.11 mmol) was dissolved in dry CH_2Cl_2 (1 mL) and stirred at room temperature during the addition of freshly distilled thionyl chloride (130 mg, 1.1 mmol). The mixture was then refluxed for 15 min, after which time the solvent was removed *in vacuo* to leave an orange oil. The oil was redissolved in dry CH_2Cl_2 (1 mL) and diluted with dry hexane (10 mL), causing an oil to separate. The solution was decanted from the oil which was washed with dry hexane, and the solvent residues were removed *in vacuo*, leaving the acid chloride as an orange glass which was used without further purification, yield 42 mg (81%).

A solution of resiniferonol 9,13,14-orthophenylacetate (24 mg, 0.052 mmol) in dry CH_2Cl_2 (0.5 mL) was stirred on ice, under a N_2 atmosphere, and triethylamine (14.6 μL, 0.1 mmol) was added. A solution of the acid chloride (36.4 mg, 0.08 mmol) in dry CH_2Cl_2 (0.5 mL) was slowly added and the reaction mixture allowed to come to room temperature with stirring over 18 h. The solvent was removed *in vacuo*, and the crude product was purified by flash column chromatography (silica gel, cyclohexane/EtOAc, 3:2). The solvent was removed from the pure fractions to leave a colorless glass, yield 31.3 mg (65%): TLC (silica gel, CH_2Cl_2/MeOH, 10:1) R_f = 0.73; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.95 (3H, d, CH_3 H$_3$-18), 1.52 (3H, s, CH_3 H$_3$-17), 1.83 (3H, br d, CH_3 H$_3$-19), 2.05 (1H, AB, H-5α), 2.14 (2H, t, 2H-12), 2.43 (1H, AB, H-5β), 2.54 (1H, m, H-11), 3.07 (2H, br m, H-10, H-8), 3.20 (2H, s, ortho ester CH_2-Ph), 3.57−3.61 (4H, br m, phenylacetyl ester ArCH_2CO, fluorenylCH_2O), 3.82 (3H, s, ArOCH_3), 4.07 (2H, br t, ArOCH$_2CH_2$NH), 4.21 (1H, d, H-14), 4.23 (1H, t, fluorenyl 5-ring CH), 4.40 (2H, m, ArOCH_2CH$_2$NH), 4.56 (2H, AB, H$_2$-20), 4.70 (2H, s, H$_2$-16), 5.29 (1H, s, 4-OH), 5.53 (1H, br t, carbamate NH), 5.87 (1H, m, H-7), 6.80−6.90 (3H, m, vanillyl ArH), 7.25−7.75 (14H, m, phenylacetyl ortho ester 5ArH, fluorenyl ArH, H-1).

9,13,14-Orthophenylacetylresiniferonyl 20-[4-(Aminoethoxy)-3-methoxyphenylacetate] (5d). 9,13,14-Orthophenylacetylresiniferonyl 20-[4-[[(fluorenylmethyloxycarbonyl) amino]ethoxy]-3-methoxyphenylacetate] (25 mg, 0.028 mmol) was dissolved in dry CH_2Cl_2 (3 mL) and stirred at room temperature. Distilled piperidine (3 mL, 30.3 mmol) was added and the reaction mixture stirred for 15 min, after which time no starting material remained by TLC. The solvents were removed *in vacuo* to leave a white solid which was purified by preparative reversed-phase HPLC (gradient 10−60% CH_3CN/H_2O). The pure fractions were evaporated, and the residue was redissolved in CH_2Cl_2, washed with water and NaCl (saturated), and then dried over Na_2SO_4 to give a colorless glass, yield 13.2 mg (69%): TLC (silica gel, CH_2Cl_2/MeOH/HOAc, 80:18:2) R_f = 0.33 (stains strongly with ninhydrin and fluorescamine); analytical reversed-phase HPLC (gradient 10−100% CH_3CN/H_2O) t_R = 12.4 min, 100% pure; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.95 (3H, d, CH_3 H$_3$-18), 1.52 (3H, s, CH_3 H$_3$-17), 1.80 (3H, br d, CH_3 H$_3$-19), 1.90−2.22 (5H, br m, H-5α, 2H-12, ArOCH$_2CH_2NH_2$), 2.30 (2H, AB, H-5β), 2.56 (1H, m, H-11), 3.04−3.10 (2H, br m, H-10, H-8), 3.20 (2H, s, ortho ester CH_2-Ph), 3.40 (2H, br m, ArOCH$_2CH_2NH_2$), 3.56 (2H, s, ArCH_2-CO), 3.85 (3H, s, ArOCH_3), 4.15 (2H, br t, ArOCH_2CH$_2$NH$_2$), 4.22 (1H, d, H-14), 4.55 (2H, m, H$_2$-20), 4.70 (2H, s, H$_2$-16), 5.88 (1H, m, H-7), 6.80−6.90 (3H, m, vanillyl ArH), 7.25−7.40 (5H, m, phenylacetyl ortho ester ArH), 7.45 (1H, m, H-1); FAB-

MS (M + 1)$^+$ 672 (100). Accurate mass (FAB MH$^+$): calcd for $C_{39}H_{46}NO_9$, 672.3173; found, 672.3170.

9,13,14-Orthophenylacetylresiniferonyl 20-Phenylacetate (5a). A solution of resiniferonol 9,13,14-orthophenylacetate (12 mg, 0.026 mmol) in dry CH_2Cl_2 (0.5 mL) was stirred on ice, under a N_2 atmosphere, and triethylamine (7.5 μL, 0.052 mmol) was added. A solution of phenylacetyl chloride (6.1 mg, 0.039 mmol) in dry CH_2Cl_2 (0.5 mL) was slowly added and the reaction mixture allowed to come to room temperature with stirring over 18 h. The solvent was removed *in vacuo*, and the crude product was purified by flash column chromatography (silica gel, cyclohexane/EtOAc, 3:1). The pure fractions were evaporated to give a colorless glass, yield 10.5 mg (70%): TLC (silica gel, CH_2Cl_2/MeOH, 25:1) R_f = 0.90; analytical reversed-phase HPLC (isocratic 80% MeOH/water) t_R = 7.37 min, >98% pure; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.95 (3H, d, C*H*$_3$ H$_3$-18), 1.62 (3H, s, C*H*$_3$ H$_3$-17), 1.83 (3H, br d, C*H*$_3$ H$_3$-19), 2.05 (1H, AB, H-5α), 2.15 (2H, m, 2H-12), 2.43 (2H, AB, H-5β), 2.56 (1H, m, H-11), 3.06 (2H, br m, H-10, H-8), 3.21 (2H, s, ortho ester C*H*$_2$Ph), 3.66 (2H, s, ArC*H*$_2$CO), 4.19 (1H, d, H-14), 4.56 (2H, AB, H$_2$-20), 4.69 (2H, s, H$_2$-16), 5.85 (1H, m, H-7), 7.15−7.45 (11H, m, phenylacetyl ortho ester ArH, C-20 ester ArH, H-1); FAB-MS (M + 1)$^+$ 583 (55). Accurate mass (FAB MH$^+$): calcd for $C_{36}H_{39}O_7$, 583.2695; found, 583.2692.

9,13,14-Orthophenylacetylresiniferonyl 20-(3-Methoxyphenylacetate) (5b). A solution of resiniferonol 9,13,14-orthophenylacetate (10 mg, 0.022 mmol) in dry CH_2Cl_2 (0.5 mL) was stirred on ice, under a N_2 atmosphere, and triethylamine (6.2 μL, 0.044 mmol) was added. A solution of the 3-methoxyphenylacetyl chloride (6.1 mg, 0.033 mmol) in dry CH_2Cl_2 (0.5 mL) was slowly added and the reaction mixture allowed to come to room temperature with stirring over 18 h. The solvent was removed *in vacuo*, and the crude product was purified by flash column chromatography (silica gel, cyclohexane/EtOAc, 3:1). The pure fractions were evaporated to give a colorless glass, yield 6.2 mg (47%): TLC (silica gel, CH_2Cl_2/MeOH, 25:1) R_f = 0.82; analytical reversed-phase HPLC (isocratic 80% MeOH/water) t_R = 5.15 min, 100% pure; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.95 (3H, d, C*H*$_3$ H$_3$-18), 1.55 (3H, s, C*H*$_3$ H$_3$-17), 1.82 (3H, br d, C*H*$_3$ H$_3$-19), 2.05 (1H, AB, H-5α), 2.13 (1H, m, H-12), 2.42 (2H, AB, H-5β), 2.55 (1H, m, H-11), 3.05 (2H, br m, H-10, H-8), 3.21 (2H, s, ortho ester C*H*$_2$Ph), 3.62 (2H, s, ArC*H*$_2$CO), 3.78 (3H, s, ArOC*H*$_3$), 4.20 (1H, d, H-14), 4.56 (2H, AB, H$_2$-20), 4.70 (2H, s, H$_2$-16), 5.86 (1H, m, H-7), 6.80−6.90 (3H, m, C-20 ester ArH$_{2,4,6}$), 7.20−7.40 (6H, m, phenylacetyl ortho ester ArH, C-20 ester ArH$_5$), 7.42 (1H, m, H-1); FAB-MS (M + 1)$^+$ 613 (60). Accurate mass (FAB MH$^+$): calcd for $C_{37}H_{41}O_8$, 613.2801; found, 613.2805.

9,13,14-Orthophenylacetylresiniferonyl 20-(3,4-Dimethoxyphenylacetate) (5c). A solution of resiniferonol 9,13,14-orthophenylacetate (10 mg, 0.022 mmol) in dry CH_2Cl_2 (0.5 mL) was stirred on ice, under a N_2 atmosphere, and triethylamine (6.2 μL, 0.044 mmol) was added. A solution of the 3,4-dimethoxyphenylacetyl chloride (7.1 mg, 0.033 mmol) in dry CH_2Cl_2 (0.5 mL) was slowly added and the reaction mixture allowed to come to room temperature with stirring over 18 h. The solvent was removed *in vacuo*, and the crude product was purified by flash column chromatography (silica gel, cyclohexane/EtOAc, 3:1). The pure fractions were evaporated to give a colorless glass, yield 5 mg (36%): TLC (silica gel, CH_2Cl_2/MeOH, 25:1) R_f = 0.60; analytical reversed-phase HPLC (isocratic 80% MeOH/water) t_R = 4.34 min, 100% pure; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.95 (3H, d, C*H*$_3$ H$_3$-18), 1.55 (3H, s, C*H*$_3$ H$_3$-17), 1.80 (3H, br d, C*H*$_3$ H$_3$-19), 2.08 (1H, AB, H-5α), 2.15 (1H, m, H-12), 2.45 (2H, AB, H-5β), 2.55 (1H, m, H-11), 3.08 (2H, br m, H-10, H-8), 3.20 (2H, s, ortho ester C*H*$_2$-Ph), 3.58 (2H, s, ArC*H*$_2$CO), 3.86 (3H, s, ArOC*H*$_3$), 3.88 (3H, s, ArOC*H*$_3$), 4.21 (1H, d, H-14), 4.58 (2H, AB, H$_2$-20), 4.70 (2H, s, H$_2$-16), 5.87 (1H, m, H-7), 6.80 (3H, m, C-20 ester ArH), 7.20−7.40 (5H, m, phenylacetyl ortho ester ArH), 7.43 (1H, m, H-1); FAB-MS (M + 1)$^+$ 643 (50). Accurate mass (FAB MH$^+$): calcd for $C_{38}H_{43}O_9$, 643.2907; found, 643.2902.

9,13,14-Orthophenylacetylresiniferonyl 20-Acetate (4a). A solution of resiniferonol 9,13,14-orthophenylacetate (12.3 mg, 0.027 mmol) in dry CH_2Cl_2 (0.5 mL) was stirred on ice, under a N_2 atmosphere, and triethylamine (5.5 μL, 0.041 mmol) was added. A solution of acetyl chloride (2 μM, 0.027 mmol) in dry CH_2Cl_2 (0.5 mL) was slowly added and the reaction mixture allowed to come to room temperature with stirring over 3 h. The solvent was removed *in vacuo*, and the crude product was purified by flash column chromatography (silica gel, cyclohexane/EtOAc, 3:1). The pure fractions were evaporated to give a colorless glass, yield 11.2 mg (83%): TLC (silica gel, cyclohexane/EtOAc, 1:1) R_f = 0.50; analytical reversed-phase HPLC (gradient 10−70% MeOH/water) t_R = 14.92 min, 100% pure; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.96 (3H, d, C*H*$_3$ H$_3$-18), 1.54 (3H, s, C*H*$_3$ H$_3$-17), 1.83 (3H, br d, C*H*$_3$ H$_3$-19), 2.09 (3H, OCOC*H*$_3$), 2.10−2.23 (1H, m, H-5α, 2H-12), 2.50−2.62 (2H, m, H-11, H-5β), 3.14 (2H, br m, H-10, H-8), 3.21 (2H, s, ortho ester C*H*$_2$Ph), 4.26 (1H, d, H-14), 4.54 (2H, AB, H$_2$-20), 4.70 (2H, s, H$_2$-16), 5.92 (1H, m, H-7), 7.20−7.40 (5H, m, phenylacetyl ortho ester ArH), 7.46 (1H, m, H-1); FAB-MS (M + 1)$^+$ 507 (100). Accurate mass (FAB MH$^+$): calcd for $C_{30}H_{35}O_7$, 507.2383; found, 507.2380.

9,13,14-Orthophenylacetylresiniferonyl 20-Nonanoate (4b). A solution of resiniferonol 9,13,14-orthophenylacetate (9 mg, 0.020 mmol) in dry CH_2Cl_2 (0.5 mL) was stirred on ice, under a N_2 atmosphere, and triethylamine (3.1 μL, 0.022 mmol) was added. A solution of the nonanoyl chloride (3.9 mg, 0.022 mmol) in dry CH_2Cl_2 (0.5 mL) was slowly added and the reaction mixture allowed to come to room temperature with stirring over 18 h. The solvent was removed *in vacuo*, and the crude product was purified by flash column chromatography (silica gel, cyclohexane/EtOAc, 4:1). The pure fractions were evaporated to give a colorless glass, yield 5 mg (36%): TLC (silica gel, CH_2Cl_2/MeOH, 10:1) R_f = 0.85; analytical reversed-phase HPLC (gradient 10−70% MeOH/water) t_R = 17.8 min, >98% pure; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.89 (3H, br t, alkyl C*H*$_3$), 0.96 (3H, d, C*H*$_3$ H$_3$-18), 1.21−1.35 (10H, env, alkyl C*H*$_2$), 1.54 (3H, s, C*H*$_3$ H$_3$-17), 1.60−1.70 (2H, br m, COCH$_2$C*H*$_2$), 1.83 (3H, br d, C*H*$_3$ H$_3$-19), 2.11−2.18 (3H, m, H-5a, 2H-12), 2.32 (2H, t, COC*H*$_2$CH$_2$), 2.50−2.62 (2H, m, H-11, H-5β), 3.13 (2H, br m, H-10, H-8), 3.22 (2H, s, ortho ester C*H*$_2$Ph), 4.26 (1H, d, H-14), 4.55 (2H, AB, H$_2$-20), 4.70 (2H, s, H$_2$-16), 5.90 (1H, m, H-7), 7.20−7.40 (5H, m, phenylacetyl ortho ester ArH), 7.46 (1H, m, H-1). FAB-MS (M + 1)$^+$ 605 (100). Accurate mass (FAB MH$^+$): calcd for $C_{37}H_{49}O_7$, 605.3478; found, 605.3474.

9,13,14-Orthoacetylresiniferonyl 20-(4-Acetoxy-3-methoxyphenylacetate). A solution of resiniferonol 9,13,14-orthoacetate[13] (4.4 mg, 0.011 mmol) and (dimethylamino)pyridine (0.14 g, 0.0013 mmol) in dry CH_2Cl_2 (0.5 mL) was stirred at room temperature, and a solution of acetylhomovanillic acid (2.72 mg, 0.013 mmol) in dry CH_2Cl_2 (0.5 mL) and a solution of DCCI (2.48 mg, 0.013 mmol) were added. The reaction mixture was stirred for 1 h, and then the solvent was removed *in vacuo*. Diethyl ether (2 mL) was added to the residue, the suspension was filtered, and the filtrate was evaporated to leave a colorless glass. The crude product was purified by preparative HPLC (isocratic 70% MeOH/H_2O), yield 7.5 mg (89%): TLC (silica gel, cyclohexane/EtOAc, 1:1) R_f = 0.36; FAB-MS (M + 1)$^+$ 595 (100).

9,13,14-Orthoacetylresiniferonyl 20-(4-Hydroxy-3-methoxyphenylacetate) (10a). 9,13,14-Orthoacetylresiniferonyl 4-acetoxy-3-methoxyphenylacetate (7 mg, 0.011 mmol) was dissolved in dry CH_2Cl_2 (1 mL) and stirred at room temperature, under a N_2 atmosphere. Pyrrolidine (28.4 mg, 0.37 mmol) in CH_2Cl_2 (0.1 mL) was added. After 70 min, no starting material remained by TLC. The solvent was evaporated *in vacuo*, and the crude product was purified by preparative HPLC (isocratic 70% MeOH/H_2O). The pure fractions were evaporated to give a colorless glass, yield 5 mg (86%): TLC (silica gel, CH_2Cl_2/MeOH, 50:1) R_f = 0.41; analytical reversed-phase HPLC (gradient 10−100% MeCN/0.1% aqueous TFA) t_R = 12.0 min, >97% pure; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.15 (3H, d, C*H*$_3$ H$_3$-18), 1.65 (3H, s, orthoacetyl C*H*$_3$), 1.74 (3H, br d, C*H*$_3$ H$_3$-19), 1.82 (3H, s, C*H*$_3$ H$_3$-17), 2.03 (1H, AB, H-5α), 2.18 (2H, m, H-12), 2.41 (2H, AB, H-5β), 2.59 (1H, m, H-11), 3.01 (1H, br m, H-10), 3.08 (1H, br m, H-8), 3.55 (2H, s, ArC*H*$_2$-CO), 3.89 (3H, s, ArOC*H*$_3$), 4.22 (1H, d, H-14), 4.54 (2H, AB, H$_2$-20), 4.88 (1H, s, H-16), 4.98 (1H, s, H-16), 5.84 (1H, m, H-7),

6.80 (3H, m, vanillyl ArH), 7.51 (1H, m, H-1). FAB-MS (M + 1)$^+$ 533 (40). Accurate mass (FAB MH$^+$): calcd for $C_{31}H_{37}O_9$, 553.2438; found, 553.2434.

Resiniferonol. Resiniferonol 9,13,14-orthophenylacetate (105 mg, 0.23 mmol) was dissolved in MeOH (70 mL), and 1 N HCl (24 mL, 24 mmol) was added. The reaction mixture was stirred for 4 h at room temperature before the addition of 1 M NaOMe solution in MeOH (30 mL, final pH = 10) and stirring for a further 30 min. After this time the reaction mixture contained only starting material and resiniferonol by HPLC. The solvent was evaporated, and the crude product was purified by preparative HPLC (MeOH/H_2O gradient, 10−70%). The pure fractions were evaporated to give a colorless glass, yield 53.7 mg (60%), as well as 37 mg (35%) of recovered starting material: TLC (silica gel, CH_2Cl_2/MeOH, 10:1) R_f = 0.16; analytical reversed-phase HPLC (gradient 10−70% MeOH/H_2O) t_R = 11.2 min, >99% pure; ^{1}H NMR (CD_3OD, 200 MHz) δ 0.94 (3H, d, CH_3 H_3-18), 1.74 (3H, br d, CH_3 H_3-19), 1.77 (3H, s, CH_3 H_3-17), 1.90−2.02, (2H, m, H_2-12), 2.33−2.35 (2H, m, AB, H-5α, H-11), 2.50 (1H, AB, H-5β), 3.16 (1H, br m, H-10), 3.42 (1H, br m, H-8), 3.96 (2H, m, H_2-20), 4.01 (1H, d, H-14), 5.05 (2H, m, H_2-16), 5.90 (1H, m, H-7), 7.52 (1H, m, H-1); FAB-MS (M + 1)$^+$ 391 (100).

Resiniferonol 14,20-Dibenzoate. Resiniferonol (54 mg, 0.14 mmol) was dissolved in dry EtOAc (6 mL), DMAP (38 mg, 0.31 mmol) was added, and the mixture was stirred at room temperature under a N_2 atmosphere. A solution of benzoic anhydride (70 mg, 0.31 mmol) in EtOAc (3 mL) was slowly added, and the reaction mixture was stirred for 18 h. TLC indicated the absence of resiniferonol but the presence of significant monobenzoylated material. Further benzoic anhydride (14 mg, 0.062 mmol) and DMAP (7.6 mg, 0.062 mmol) were added, and the solution was stirred for a further 18 h. After this time TLC indicated that the reaction mixture was mainly dibenzoylated material. The solution was washed with water and NaCl (saturated) and dried over $MgSO_4$. The crude product was purified by flash column chromatography (silica gel, EtOAc/cyclohexane, 1:4) to give a colorless glass, yield 40.7 mg (51.5%): TLC (silica gel, EtOAc/cyclohexane, 1:1) R_f = 0.35; ^{1}H NMR (CD_3OD, 200 MHz) δ 1.05 (3H, d, CH_3 H_3-18), 1.70 (1H, d, H-12α), 1.75 (3H, s, CH_3 H_3-17), 1.86 (3H, br d, CH_3 H_3-19), 2.25−2.46 (3H, m, H-5α, H-11, H-12β), 2.64 (1H, AB, H-5β), 3.11 (1H, br m, H-8), 4.02 (1H, br m, H-10), 4.68 (2H, m, H_2-20), 5.12 (1H, s, H-16), 5.18 (1H, 2, H-16), 5.77 (1H, br d, H-7), 5.87 (1H, s, H-14), 7.34−7.66 (7H, m, benzoyl Ar$H_{3,4,5}$, H-1), 7.92−8.08 (4H, m, benzoyl Ar$H_{2,6}$); FAB-MS (M + 1)$^+$ 573 (30).

9,13,14-Orthobenzoylresiniferonol 20-Benzoate. A solution of resiniferonol 14,20-dibenzoate (20.6 mg, 0.036 mmol) in dry dichloroethane (20 mL) was added by syringe to a dry flask containing anhydrous $CaCl_2$ (206 mg, 1.86 mmol) and anhydrous toluenesulfonic acid (6 mg, 0.036 mmol). The reaction mixture was heated to 80 °C for 1 h after which no starting material remained. The precipitate was removed by filtration, the solvent was evaporated, and the crude product was purified by preparative HPLC (isocratic 75% MeOH/H_2O). The pure fractions were evaporated *in vacuo* to give a colorless glass, yield 19.6 mg (98%): TLC (silica gel, EtOAc/cyclohexane, 1:1) R_f = 0.62; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.28 (3H, d, CH_3 H_3-18), 1.75 (1H, d, H-12α), 1.83 (3H, s, CH_3 H_3-17), 1.86 (3H, br m, CH_3 H_3-19), 2.20−2.38 (2H, m, H-5α, H-12β), 2.68 (1H, AB, H-5β), 2.77 (1H, m, H-11), 3.31 (2H, br m, H-8, H-10), 4.53 (1H, d, H-14), 4.78 (2H, AB, H_2-20), 4.92 (1H, s, H-16), 5.07 (1H, s, H-16), 6.10 (1H, br m, H-7), 7.38−8.04 (11H, m, benzoyl, orthobenzoyl ArH, H-1).

9,13,14-Orthobenzoylresiniferonol. 9,13,14-Orthobenzoylresiniferonol 20-benzoate (19.6 mg, 0.035 mmol) was dissolved in dry MeOH (10 mL) and stirred at room temperature under a N_2 atmosphere. A solution of NaOMe (390 μL of 1 M solution, 0.39 mmol) in dry methanol was added, and the reaction mixture was stirred for 1 h, after which time no starting material remained by TLC. The crude product was purified by preparative HPLC (isocratic 65% MeOH/H_2O), and the pure fractions were evaporated *in vacuo* to give a colorless glass, yield 14.5 mg (91%): TLC (silica gel, EtOAc/cyclohexane, 1:1) R_f = 0.19; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.25 (3H, d, CH_3 H_3-18), 1.72 (1H, d, H-12α), 1.84 (3H, s, CH_3 H_3-17), 1.86 (3H, br m, CH_3 H_3-19), 2.20−2.38 (2H, m, H-5α, H-12β), 2.59 (1H, AB, H-5β), 2.76 (1H, m, H-11), 3.26 (2H, br m, H-8, H-10), 4.08 (2H, s, H_2-20), 4.49 (1H, d, H-14), 4.91 (1H, m, H-16), 5.06 (1H, s, H-16), 5.92 (1H, br m, H-7), 7.40 (3H, m, orthobenzoyl Ar$H_{3,4,5}$), 7.62 (1H, m, H-1), 7.75 (2H, m, orthobenzoyl Ar$H_{2,6}$).

9,13,14-Orthobenzoylresiniferonyl 20-(4-Acetoxy-3-methoxyphenylacetate). 9,13,14-Orthobenzoylresiniferonol (14 mg, 0.031 mmol) was dissolved in dry CH_2Cl_2 (3 mL) and stirred at room temperature under a N_2 atmosphere. A solution of DCCI (7.2 mg, 0.034 mmol) and DMAP (0.42 mg, 0.0034 mmol) in CH_2Cl_2 (0.5 mL) was added followed by a solution of acetylhomovanillic acid (7.8 mg, 0.034 mmol) in CH_2Cl_2 (0.5 mL). The reaction mixture was stirred for 1 h at room temperature, after which time no starting material remained by TLC. The solvent was evaporated *in vacuo*, and the residue was suspended in diethyl ether, the solid removed by filtration, and the filtrate evaporated *in vacuo* to leave the crude product which was purified by preparative HPLC (isocratic 73% MeOH/H_2O); the pure fractions were evaporated *in vacuo* to give a colorless glass, yield 14 mg (66%): TLC (silica gel, EtOAc/cyclohexane, 1:1) R_f = 0.42; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.23 (3H, d, CH_3 H_3-18), 1.70 (1H, d, H-12a), 1.82 (6H, br s, CH_3 H_3-17, H_3-19), 2.05 (1H, d, H-5α), 2.20−2.38 (2H, s, m, ArOCOCH_3, H-5β, H-12β), 2.76 (1H, m, H-11), 3.15 (1H, br m, H-8), 3.22 (1H, br m, H-10), 3.58 (2H, s, ArCH_2CO), 3.81 (3H, s, ArOCH_3), 4.44 (1H, d, H-14), 4.54 (2H, s, H_2-20), 4.89 (1H, m, H-16), 5.05 (1H, s, H-16), 5.94 (1H, br m, H-7), 6.83−7.02 (3H, m, vanillyl ArH), 7.40 (3H, m, orthobenzoyl Ar$H_{3,4,5}$), 7.55 (1H, m, H-1), 7.75 (2H, m, orthobenzoyl Ar$H_{2,6}$).

9,13,14-Orthobenzoylresiniferonyl 20-(4-Hydroxy-3-methoxyphenylacetate) (10b). 9,13,14-Orthobenzoylresiniferonyl 20-(4-acetoxy-3-methoxyphenylacetate) (8 mg, 0.012 mmol) was dissolved in dry CH_2Cl_2 (1 mL) and stirred at room temperature under N_2. A solution of pyrrolidine (50 μL, 0.60 mmol) in CH_2Cl_2 (0.5 mL) was added and the reaction mixture was stirred for 90 min, after which time no starting material remained by TLC. The solvent was removed *in vacuo*, the crude product was purified by preparative HPLC (isocratic 70% MeOH/H_2O), and the pure fractions were evaporated *in vacuo* to give a colorless glass, yield 7 mg (93%): TLC (silica gel, EtOAc/cyclohexane, 1:1) R_f = 0.32; HPLC (isocratic 70% MeOH/H_2O) t_R = 10.5 min, 100% pure; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.25 (3H, d, CH_3 H_3-18), 1.70 (1H, d, H-12α), 1.82 (6H, br s, CH_3 H_3-17, H_3-19), 2.05 (1H, d, H-5α), 2.30 (1H, m, H-12β), 2.44 (1H, AB, H-5β), 2.72 (1H, m, H-11), 3.15 (1H, br m, H-8), 3.24 (1H, br m, H-10), 3.52 (2H, s, ArCH_2CO), 3.81 (3H, s, ArOCH_3), 4.43 (1H, d, H-14), 4.53 (2H, AB, H_2-20), 4.91 (1H, m, H-16), 5.06 (1H, s, H-16), 5.92 (1H, br m, H-7), 6.70−6.85 (3H, m, vanillyl ArH), 7.35−7.44 (3H, m, orthobenzoyl Ar$H_{3,4,5}$), 7.60 (1H, m, H-1), 7.75 (2H, m, orthobenzoyl Ar$H_{2,6}$); FAB-MS (M + 1)$^+$ 615 (20). Accurate mass (FAB MH$^+$): calcd for $C_{37}H_{39}O_9$, 615.2594; found, 615.2590.

9,13,14-Orthophenylacetyl-3β-hydroxyresiniferonyl 20-(4-Hydroxy-3-methoxyphenylacetate) (11b). Resiniferatoxin (11.3 mg, 0.018 mmol) was dissolved in absolute ethanol (1 mL) and stirred at room temperature. $NaBH_4$ (3.4 mg, 0.089 mmol) was added and the reaction mixture stirred for 2 h. After this time no starting material remained by TLC, and so AcOH (15 μM) was added and the solvent removed *in vacuo*. The residue was redissolved in CH_2Cl_2, washed with water and saturated NaCl, and dried over Na_2SO_4. The solvent was removed *in vacuo* to leave a glass which was purified by preparative HPLC (isocratic 75% MeOH/H_2O). The pure fractions were evaporated to give a colorless glass, yield 6.8 mg (60%): TLC (silica gel, EtOAc/cyclohexane, 1:1) R_f = 0.24; analytical reversed-phase HPLC (gradient 10−100% MeCN/0.1% aqueous TFA) t_R = 17.2 min, 100% pure; ^{1}H NMR (CD_3OD, 200 MHz) δ 0.98 (3H, d, CH_3 H_3-18), 1.42 (1H, d, H-12α), 1.51 (3H, s, CH_3 H_3-17), 1.72 (3H, br d, CH_3 H_3-19), 2.10 (1H, AB, H-5α), 2.16 (1H, m, H-12β), 2.40 (1H, AB, H-5β), 2.65 (1H, m, H-11), 2.69 (1H, br m, H-10), 3.02 (1H, br m, H-8), 3.12 (2H, s, ortho ester CH_2Ph), 3.54 (2H, AB, ArCH_2CO), 3.80 (3H, s, ArOCH_3), 3.89 (1H, br, H-3), 4.15 (1H, d, H-14), 4.55 (2H, AB, H_2-20), 4.66 (1H, s, H-16), 4.71 (1H, s, H-16), 5.48 (1H, br m, H-1), 5.83 (1H, m, H-7), 6.70−6.83 (3H, m, vanillyl ArH),

7.15−7.36 (5H, m, phenylacetyl ortho ester ArH); FAB-MS (M + 1)$^+$ 631 (32). Accurate mass (FAB MH$^+$): calcd for $C_{37}H_{43}O_9$, 631.2907; found, 631.2903.

3β-Epimer was assigned by NOE difference NMR spectroscopy: H-10 irradiated (δ 2.70); NOE-H-5α, H-3; H-3 irradiated (δ 3.89); NOE-H-10, H-5α, 3H-19; H-8 identified by NOE from irradiation of H-14 (δ 4.18)-NOE to H-8, H-7, 3H-17.

9,13,14-Orthophenylacetyl-4β-methoxyresiniferonol. 9,-13,14-Orthophenylacetylresiniferonyl 20-acetate, **4a** (50 mg, 0.099 mmol), in dry DMF (0.3 mL) was stirred under a N_2 atmosphere with Ag_2O (35 mg, 0.15 mmol). A solution of methyl iodide (100 μL, 1.62 mmol) in DMF (50 μL) was added, and the reaction mixture was stirred for 18 h at room temperature. The solvent was evaporated, and methanol (0.5 mL) was added to the residue. The insoluble material was removed by filtration and the acetyl protecting group removed from the crude product *in situ* by transesterification by the addition of a methanolic solution of NaOMe (1.1 mmol). After stirring for 1 h at room temperature, the solvent was removed *in vacuo* and the product was purified by preparative HPLC (isocratic 72% MeOH/H_2O); the pure fractions were evaporated to give a colorless glass, yield 32 mg (67%): TLC (silica gel, EtOAc/cyclohexane, 1:1) R_f = 0.27; analytical reversed-phase HPLC (isocratic 70% MeOH/H_2O) t_R = 11.2 min, 100% pure; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.99 (3H, d, CH_3 H$_3$-18), 1.53 (3H, s, CH_3 H$_3$-17), 1.56 (1H, d, H-12α), 1.80 (3H, br d, CH_3 H$_3$-19), 2.00 (1H, AB, H-5α), 2.12 (1H, m, H-12β), 2.62 (1H, m, H-11), 2.93 (1H, br m, H-8), 3.02 (1H, AB, H-5β), 3.12 (1H, m, H-10), 3.20 (2H, s, ortho ester CH_2Ph), 3.32 (3H, s, 4-OCH_3), 4.10 (2H, m, H$_2$-20), 4.24 (1H, d, H-14), 4.70 (2H, s, H$_2$-16), 5.86 (1H, m, H-7), 7.20−7.40 (6H, m, phenylacetyl ortho ester ArH, H-1); FAB-MS (M + 1)$^+$ 479 (100).

9,13,14-Orthophenylacetyl-4β-methoxyresiniferonyl 20-(4-Hydroxy-3-methoxyphenylacetate) (11a). 9,13,14-Orthophenylacetyl-4β-methoxyresiniferonol (27 mg, 0.056 mmol) was dissolved in CH_2Cl_2 (2.5 mL), and to this were added DCCI (12.7 mg, 0.062 mmol) and DMAP (0.75 mg, 0.0062 mmol). The solution was stirred at room temperature, and acetylhomovanillic acid (13.9 mg, 0.062 mmol) in CH_2Cl_2 (0.5 mL) was added. Stirring was continued for 2 h, after which time the solution was washed with 1 N HCl, water, and then NaCl (saturated) and dried over Na_2SO_4. The crude product was evaporated *in vacuo* to give a colorless glass which was >95% pure by TLC (silica gel, EtOAc/cyclohexane, 1:1; R_f = 0.43). This material, in CH_2Cl_2 (2 mL), was deprotected without further purification by addition of pyrrolidine (200 μL, 2.2 mmol) and stirring at room temperature for 30 min. The solution was washed with 1 N HCl, water, and then NaCl (saturated) and dried over Na_2SO_4. The crude product was purified by preparative HPLC (isocratic 73% MeOH/H_2O), and the pure fractions were evaporated to give a colorless glass, yield 20.7 mg (57%): TLC (silica gel, EtOAc/cyclohexane, 1:1) R_f = 0.44; analytical reversed-phase HPLC (isocratic 75% MeOH/H_2O) t_R = 8.6 min, >98% pure; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.97 (3H, d, CH_3 H$_3$-18), 1.52 (3H, s, CH_3 H$_3$-17), 1.58 (1H, d, H-12α), 1.75−1.82 (4H, br m, CH_3 H$_3$-19, H-5α), 2.09 (1H, m, H-12β), 2.57 (1H, m, H-11), 2.87−2.94 (1H, br m, d, H-8, H-5β), 3.04 (1H, br m, H-10), 3.21 (2H, s, ortho ester CH_2-Ph), 3.24 (3H, s, 4-OCH_3), 3.57 (2H, AB, ArCH_2CO), 3.90 (3H, s, ArOCH_3), 4.19 (1H, d, H-14), 4.58 (2H, AB, H$_2$-20), 4.71 (2H, d, H$_2$-16), 5.86 (1H, br m, H-7), 6.75−6.87 (3H, m, vanillyl ArH), 7.20−7.40 (6H, m, phenylacetyl ortho ester ArH, H-1); FAB-MS (M + 1)$^+$ 643 (50). Accurate mass (FAB MH$^+$): calcd for $C_{38}H_{43}O_9$, 643.2907; found, 643.2903.

Phorbol Analogues: 12-Deoxyphorbol 13-Phenylacetate 20-(4-Hydroxy-3-methoxyphenylacetate) (8).[12] 12-Deoxyphorbol 13-phenylacetate (25 mg, 0.054 mmol) was dissolved in dry DMF (0.5 mL), and to this were added triethylamine (12 μL, 0.096 mmol) and 2-(fluoromethyl)-pyridinium tosylate (29.4 mg, 0.105 mmol) in DMF (0.2 mL). The reaction mixture was stirred at room temperature, under N_2, for 30 min before the addition of further triethylamine (19.2 μL, 0.146 mmol) and homovanillic acid (28 mg, 0.146 mmol) in DMF (0.2 mL). The reaction mixture was heated to 60 °C for 2 h, after which time the solvent was removed *in vacuo* and purified by flash column chromatography (silica gel, EtOAc/cyclohexane, 1:2). The pure fractions were evaporated *in vacuo* to give a colorless glass, yield 4 mg (12%): TLC (silica gel, CH_2Cl_2/MeOH, 25:1) R_f = 0.21; analytical reversed-phase HPLC (gradient 10−100% CH_3CN/0.1% aqueous TFA) t_R = 12.4 min, >98% pure; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.66 (1H, d, H-14), 0.85 (3H, d, CH_3 H$_3$-18), 1.02 (3H, s, CH_3 H$_3$-16), 1.04 (3H, s, CH_3 H$_3$-17), 1.75 (3H, d, CH_3 H$_3$-19), 2.05 (2H, m, H-12), 2.26 (1H, AB, H-5α), 2.40 (1H, AB, H-5β), 2.90 (1H, m, H-8), 3.18 (1H, m, H-10), 3.52 (2H, s, ArCH_2CO), 3.61 (2H, s, ArCH_2-CO), 3.78 (3H, s, ArOCH_3), 4.46 (2H, m, H$_2$-20), 5.34 (1H, br s, ArOH), 5.60 (1H, br m, H-7), 6.73−6.81 (3H, m, vanillyl ArH), 7.25−7.35 (5H, m, phenylacetyl ester ArH), 7.56 (1H, s, H-1); FAB-MS (M + 1)$^+$ 631 (7). Accurate mass (FAB MH$^+$): calcd for $C_{37}H_{43}O_9$, 631.2907; found, 631.2903.

Phorbol 12,13-Diacetate 20-(4-Hydroxy-3-methoxyphenylacetate) (9a). Phorbol 12,13-diacetate (90 mg, 0.2 mmol) was dissolved in dry DMF (1 mL), and to this were added triethylamine (46 μL, 0.36 mmol) and 2-(fluoromethyl)pyridinium tosylate (109 mg, 0.39 mmol) in DMF (0.2 mL). The reaction mixture was stirred at room temperature, under N_2, for 30 min before the addition of further triethylamine (71 μL, 0.54 mmol) and homovanillic acid (99 mg, 0.54 mmol) in DMF (0.2 mL). The reaction mixture was heated to 60 °C for 2 h, after which time the solvent was removed *in vacuo* and purified by flash column chromatography (silica gel, EtOAc/cyclohexane, 1:2). The pure fractions were evaporated *in vacuo* to give a colorless glass, yield 29 mg (24%): TLC (silica gel, CH_2Cl_2/MeOH, 25:1) R_f = 0.41; analytical reversed-phase HPLC (gradient 10−100% CH_3CN/0.1% aqueous TFA) t_R = 12.8 min, 100% pure; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.76 (3H, d, CH_3 H$_3$-18), 1.00 (1H, d, H-14), 1.12 (3H, s, CH_3 H$_3$-16), 1.14 (3H, s, CH_3 H$_3$-17), 1.68 (3H, d, CH_3 H$_3$-19), 2.02 (6H, s, 2 × OCOCH_3), 2.22 (1H, AB, H-5α), 2.42 (1H, AB, H-5β), 2.94 (1H, m, H-8), 3.08 (1H, m, H-10), 3.52 (2H, s, ArCH_2CO), 3.74 (3H, s, ArOCH_3), 4.44 (2H, AB, H$_2$-20), 5.07 (1H, br s, OH), 5.32 (1H, d, H-12), 5.54 (1H, br m, H-7), 5.90 (1H, br s, OH), 6.62−6.81 (3H, m, vanillyl ArH), 7.50 (1H, s, H-1), 8.88 (1H, br s, ArOH). FAB-MS (M + 1)$^+$ 613 (12). Accurate mass (FAB MH$^+$): calcd for $C_{33}H_{41}O_{11}$, 613.2649; found, 613.2645.

Phorbol 12,13-Didecanoate 20-(4-Hydroxy-3-methoxyphenylacetate) (9b). This compound was synthesized by an analogous method to that described for phorbol-12,13-diacetate 20-(4-hydroxy-3-methoxyphenylacetate) from phorbol 12,13-didecanoate and purified by flash column chromatography (silica gel, EtOAc/cyclohexane, 1:2). The pure fractions were evaporated *in vacuo* to give a colorless glass, yield 5.5 mg (17.7%): TLC (silica gel, EtOAc/cyclohexane, 1:1) R_f = 0.49; analytical reversed-phase HPLC (gradient 10−100% CH_3CN/0.1% aqueous TFA) t_R = 13.6 min, >98% pure; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.88 (9H, m, CH_3 H$_3$-18, 2 × decanoate CH_3), 0.94 (1H, d, H-14), 1.19 (3H, s, CH_3 H$_3$-16), 1.21 (3H, s, CH_3 H$_3$-17), 1.77 (3H, d, CH_3 H$_3$-19), 2.32 (5H, m, 2 × OCOCH_2-CH$_2$, H-5α), 2.45 (1H, AB, H-5β), 3.15−3.19 (2H, m, H-8, H-10), 3.55 (2H, s, ArCH_2CO), 3.88 (3H, s, ArOCH_3), 4.46 (2H, AB, H$_2$-20), 5.39 (1H, d, H-12), 5.54 (1H, br s, OH), 5.62−5.65 (2H, br m, s, H-7, OH), 6.73−6.82 (3H, m, vanillyl ArH), 7.56 (1H, s, H-1); FAB-MS (M + 1)$^+$ 838 (10). Accurate mass (FAB MH$^+$): calcd for $C_{49}H_{73}O_{11}$, 837.5153; found, 837.5150.

Molecular Modeling. Studies on compounds **2**, **8**, and **12** were performed on an Alliant Fx2800 computer using a Silicon Graphics workstation as the graphics display unit. The molecules were constructed using a proprietary modeling package, Draw, and the structures optimized using the algorithms of the molecular mechanics program Minimax.[23] The conformational space available to these compounds was explored using molecular dynamic (MD) simulations, accomplished using the Insight/Discover suite of programs.[24] Thus each compound was submitted to the following protocol: A starting structure was energy minimized using the CVFF force field and the conjugate gradient method until the rms derivative of the energy was below 0.01 kcal/mol/Å. The system was then brought to equilibrium, over 1 ps at a temperature of 300 K, before a molecular simulation study spanning a further 250 ps (at 300 K) was undertaken. Snap shots taken at 1 ps intervals were minimized using the

optimization criteria outlined above. For each compound, the resulting minimized structures were analyzed by superimposition of the diterpene 7-membered ring moiety.

Biology. *In Vitro* Assays: Stimulation of $^{45}Ca^{2+}$ Uptake into Dorsal Root Ganglion (DRG) Neurons. The uptake and accumulation of $^{45}Ca^{2+}$ by capsaicin analogues were studied in neonatal rat cultured spinal sensory neurons by the method described in detail by Wood *et al.*[5] In brief, spinal (dorsal root) ganglia were dissected aseptically from newborn rats and incubated sequentially at 37 °C for 30 min with collagenase (Boeringer Mannheim) followed by 30 min in 2.5 mg/mL trypsin (Worthington), both enzymes made up in Ham's F-14 medium. The ganglia were then washed in medium supplemented with 10% horse serum and the cells dissociated by trituration through a Pasteur pipet. The cells were collected by centrifugation and resuspended in Ham's F-14 medium with 10% horse serum plus 1 μg/mL nerve growth factor. The neuronal preparation was plated onto poly-D-ornithine Terasaki plates (Flow Laboratories) at a density of 1000 neurons/well. Cultures were incubated at 37 °C in a humidified incubator gassed with 3% CO_2 in air. After the cells had adhered, 10^{-4} M cytosine arabinoside, a mitotic inhibitor, was added to the culture for 48 h to kill the dividing non-neuronal cells.

$^{45}Ca^{2+}$ uptake assays were made on 3-7 day old cultures. The Terasaki plates were washed four times with calcium-free Hank's balanced salt solution (BSS) buffered with 10 mM HEPES (pH = 7.4). Excess medium was drained from the plate and then 10 μL of remaining medium removed from the individual wells; 10 μL of medium containing the test concentration of compound plus 10 μCi/mL $^{45}Ca^{2+}$ (Amersham) was added to each well. All media contained 1% dimethyl sulfoxide (DMSO) to keep the compounds in solution. The neurons were incubated at room temperature for 10 min and then the Terasaki plates washed six times in BSS and dried in an oven; 10 μL of 0.3% sodium dodecyl sulfate was added to each well to dissolve the cells and extract the $^{45}Ca^{2+}$. The contents of each well were transferred to scintillation vials and counted in 1 mL of Beckman CP scintillation fluid. In all experiments one group of replicates was treated with medium alone to estimate the background uptake.

EC_{50} values (the concentration of drug necessary to produce 50% of the maximal response) were estimated with at least six replicates at each concentration. Each compound was tested in two or more independent experiments. Data were fitted with a sigmoidal function of the form:

$$\text{total uptake} = a/(1 + (EC_{50}/\text{conc})^b) + c$$

where a = the maximum evoked uptake, b = the slope factor, and c = the background uptake in the absence of compound. Results are given as mean ± SEM.

Displacement of [^{3}H]RTX Binding from DRG Membranes. Binding assays were carried out as described in detail by Szallasi *et al.*[16] Briefly, female Sprague−Dawley rats (250−300 g) were sacrificed by decapitation under CO_2 anesthesia; the cervical and upper thoracic DRG were removed and disrupted using a Polytron tissue homogenizer in ice-cold buffer (pH = 7.4) containing (in mM) KCl, 5; NaCl, 5.8; $MgCl_2$, 2; $CaCl_2$, 0.75; sucrose, 137 and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 10. Tissue homogenates were washed twice in the same buffer, and the particulate fraction was stored at −70 °C; 25−30 μg aliquots of the DRG particulate fraction in 0.5 mL of the above buffer containing 0.25 mg/mL bovine serum albumin (Cohn fraction V; Sigma Chemical Co., St. Louis, MO), a carrier protein included to stabilize RTX in aqueous solution, were incubated in triplicate with [^{3}H]RTX and nonradioactive ligands at 37 °C for 30 min. Nonspecific binding was determined in the presence of 100 nM nonradioactive RTX. Tubes were kept on ice while the additions were made. After the binding reaction had been terminated by chilling the assay mixture on ice, 100 μg of bovine α_1-acid glycoprotein (Sigma) in 50 μL of Dulbecco's phosphate-buffered saline was added to reduce nonspecific binding. Bound and free [^{3}H]RTX were then separated by pelleting the membranes in a Beckman 12 microfuge; a 200 μL aliquot of the supernatant was removed to determine free [^{3}H]RTX concentration, the remainder of the supernatant was removed by aspiration, the tip of the microfuge tube containing the pelleted membranes was cut off with a razor blade after the pellet had been carefully dried with the tip of a rolled kimwipe, and the bound radioactivity was determined by scintillation counting. Specific dpm ranged from approximately 50 dpm at 6 pM [^{3}H]-RTX to 250 dpm at 50 pM [^{3}H]RTX.

Binding data from saturation experiments using increasing concentrations of radioactive ligand were analyzed by computer fit to the Hill equation:

$$B = (B_{max}L_H^n)/(K_d^n + L_H^n) \quad (1)$$

where B is the concentration of the receptor−ligand complex, B_{max} is the maximum binding capacity, L_H is the concentration of the radioactive ligand, K_d is the concentration of ligand required to occupy one-half of the receptors, and n is the cooperativity index, also known as the Hill coefficient. Equation 1 predicts that the plot of the specific binding vs ligand concentration will be a hyperbola if the binding sites are independent of each other ($n = 1$) but will be sigmoidal if there is positive cooperativity among receptors ($n > 1$).

Binding data from experiments in which [^{3}H]RTX was displaced by increasing concentrations of nonradioactive ligands were analyzed by the modified Hill equation:[25]

$$B = ((B_{max}(L_H + L_c)^n)/(K_d^n + (L_H + L_c)^n)) \times ((L_H/(L_H + L_c)))$$

in which L_c is the concentration of the nonradioactive ligand. This equation describes a sigmoidal competition curve if $n = 1$. In contrast, if there is positive cooperativity among binding sites and $L_H < L_c$ (low receptor occupancy), the equation predicts that low concentrations of nonradioactive ligand will enhance rather than inhibit binding; the resulting competition curve will be distorted accordingly. Computer analysis was performed on an IBM PC using the program Fit P. Results are given as mean ± SEM from three independent experiments.

Supporting Information Available: Tables for HPLC gradient programs (2 pages). Ordering information is given on any current masthead page.

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