Simplified Tetrandrine Congeners as Possible Antihypertensive Agents with a Dual Mechanism of Action

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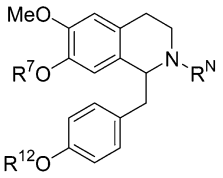
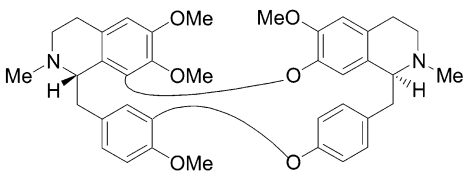
A series of *O*- and/or *N*-substituted derivatives of (±)-coclaurine (**1a**) were synthesized as simplified structural mimics of the antihypertensive alkaloid tetrandrine (**2**) and assayed for binding to brain cortical sites labeled with the α_1-adrenergic radioligand [^{3}H]prazosin or the calcium channel radioligand [^{3}H]-diltiazem. The introduction of *O*-benzyl groups on the coclaurine molecule, which exhibits only adrenergic antagonist activity, led to the appearance of calcium channel blocking activity comparable to that of tetrandrine while retaining adrenolytic activity in the same concentration range. Contraction of aortal rings with noradrenaline or KCl was relaxed more potently by some of these coclaurine derivatives than by tetrandrine, suggesting leads for the development of novel antihypertensive drugs with a dual mechanism of action.

Bisbenzylisoquinoline (BBIQ) or biscoclaurine alkaloids make up an extensive family of natural products characterized by the presence of two monomeric 1-benzyl-1,2,3,4-tetrahydroisoquinoline (BTHIQ) moieties structurally and biosynthetically related to coclaurine (**1a**).[1] In the larger, "tail-to-tail" subfamily, these halves are joined by a biphenyl ether or C−C bond between the benzyl groups and, usually, one or two extra "head-to-head" ether linkages between the isoquinoline benzene rings. Many of these alkaloids have been studied in biological systems, where they exhibit a wide range of effects including hypotensive, antiinflammatory, antiarrhythmic, bactericidal, and muscle relaxant.[2]



1 and derivatives

Tetrandrine (**2**) is the major BBIQ of "han fangji" (*Stephania tetrandra,* Menispermaceae) and is the most characteristic active constituent of this Chinese herbal remedy, which has been used for centuries in the treatment of hypertension.[3] A number of BBIQs have been compared with tetrandrine and in most cases have been found to be less effective as smooth muscle relaxants or L-type calcium channel blockers.[4−7] Within this set of BBIQs, higher smooth muscle relaxant potencies have been ascribed to the presence of two biphenyl ether linkages, the (1*S*,1′*S*) configuration, and extensive *N*- and *O*-methylation.[4,6,7] Although the smooth muscle relaxant and hypotensive action of tetrandrine is attributed to the selective blockade of calcium channels,[5,8,9] its interaction with α_1-adrenergic receptors, which also modulate calcium channels, may be significant.[10] These data suggest tetrandrine as a lead for the development of antihypertensive drugs with a dual mechanism of action.



2

Natural BBIQs, though very numerous, are seldom abundant plant metabolites, and their unsystematic structural variations weaken any argument regarding their structure−activity relationships. Their macrocyclic nature, with very specific bonding between the "eastern" and "western" regions and a stereogenic center in each, also makes their synthesis unattractive from a medicinal chemical viewpoint. On the other hand, it seems reasonable to predict that simplified, more accessible congeners might display similar pharmacological activities. In this sense, the monomeric BTHIQ coclaurine (**1a**) may serve as a template to which lipophilic substituents can be attached to mimic the structure of either half of the tetrandrine molecule. This paper describes the preparation and preliminary pharmacological screening of an extensive series of coclaurine derivatives. In these, one or both phenolic functions have been methylated or benzylated, the benzyl groups serving as an approach to the aromatic rings of the "western" half of the tetrandrine molecule. In most cases, the basic nitrogen atom has been substituted with methyl, ethyl, or propyl groups to assess the relevance of *N*-alkylation to the adrenergic and calcium channel blocking components of the smooth muscle relaxant effects of tetrandrine.

Coclaurine (**1a**) and its *O*-benzylated and/or *O*-methylated derivatives were prepared by the classical Bischler−Napieralski 3,4-dihydroisoquinoline synthesis and subsequent reduction of the intermediates with $NaBH_4$,[11−13] followed in most cases by *N*-alkylation. *N*-Methylation was best carried out with aqueous formaldehyde and $NaBH_4$,[11,12,14]

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Table 1. Coclaurine and Derivatives Discussed in this Paper[a]

compound	R^7	R^{12}	R^N
1a	H	H	H
1b	H	H	CH_3
1c	H	H	CH_2CH_3
3a	CH_3	H	H
3b	CH_3	H	CH_3
3c	CH_3	H	CH_2CH_3
4a	H	CH_3	H
4b	H	CH_3	CH_3
5a	CH_3	CH_3	H
5b	CH_3	CH_3	CH_3
6a)	Bn	Bn	H
6b	Bn	Bn	CH_3
6c	Bn	Bn	CH_2CH_3
6d	Bn	Bn	$(CH_2)_2CH_3$
7a	Bn	H	H
7b	Bn	H	CH_3
8a	Bn	CH_3	H
8b	Bn	CH_3	CH_3
9a	H	Bn	H

[a] Bn ≅ $C_6H_5CH_2$−.

Table 2. Displacement of [^{3}H]Prazosin and [^{3}H]Diltiazem from Rat Brain Cortical Sites by Coclaurine and Derivatives[a]

compound	[^{3}H]prazosin pK_i	[^{3}H]diltiazem pK_i
1a	5.97 ± 0.12	≈30%
1b	5.63 ± 0.07	4.64 ± 0.08
1c	5.29 ± 0.07	4.86 ± 0.58
3a	5.23 ± 0.12	N.D.
3b	5.14 ± 0.03	5.02 ± 0.05
3c	5.07 ± 0.09	4.51 ± 0.04
4a	4.92 ± 0.08	4.49 ± 0.04
4b	5.29 ± 0.12	4.90 ± 0.08
5b	5.10 ± 0.07	5.38 ± 0.05
6a	5.02 ± 0.04	4.61 ± 0.31
6b	5.12 ± 0.09	5.74 ± 0.11
6d	5.05 ± 0.01	5.75 ± 0.22
7a	5.49 ± 0.07	5.60 ± 0.35
7b	5.49 ± 0.05	5.70 ± 0.25
8a	5.34 ± 0.06	N.D.
8b	5.73 ± 0.03	5.82 ± 0.10
9a	5.69 ± 0.16	5.57 ± 0.28
tetrandrine (**2**)	6.16[15]	6.13 ± 0.22

[a] pK_i = −log(K_i); K_i = inhibition constant (μM) of each alkaloid for the binding of [^{3}H]prazosin or [^{3}H]diltiazem to their specific sites in rat brain cortical membranes; all binding experiments were carried out 4−6 times. N.D.: not determined.

and *N*-ethylation and *N*-propylation were performed by treating the secondary amines with ethyl iodide or propyl bromide in acetonitrile containing $NaHCO_3$, respectively. 12-*O*-Benzylcoclaurine (**9a**) was prepared conveniently by selective debenzylation of 7,12-*O*,*O*′-dibenzylcoclaurine (**6a**) with $SnCl_4$.[15]

The affinities of all the coclaurine derivatives for α_1-adrenergic receptors were assessed in binding studies in rat brain cortical homogenates using the subtype nonselective radioligand [^{3}H]prazosin. A subset of these compounds was assayed for affinity for the benzothiazepine modulatory site of the L-type Ca^{2+} channel displacing [^{3}H]diltiazem, and eight of them were selected for functional testing as α_1-adrenergic antagonists or Ca^{2+} channel blockers in rat thoracic aorta, contracted with noradrenaline or by depolarization with KCl, respectively.[10] The results were compared with available data for coclaurine itself and for tetrandrine.

Results and Discussion

The BTHIQ derivatives prepared in this study are listed in Table 1. For the sake of clarity, compound numbers followed by a letter "a" always correspond to the secondary amines, and by "b", "c", or "d" to the *N*-methylated, -ethylated, or -propylated analogues. Their ^{1}H NMR spectra were recorded and analyzed to establish their conformational preferences. Earlier ^{1}H NMR studies on BTHIQs suggested that the preferred conformation of the *N*-unsubstituted secondary amines has the pendent benzyl group pointing away from the tetrahydroisoquinoline benzene ring, while *N*-methylation leads to a preferentially folded conformation in which the benzyl group shields H-8 and the protons of the methoxyl group bonded to C-7, when such a group is present.[16−18] The "western" half of head-to-head, tail-to-tail BBIQs is held in an extended conformation, while the "eastern" half adopts a folded conformation, as is clearly seen in molecular models and corroborated by ^{1}H and ^{13}C NMR results.[19] Analysis of the ^{1}H NMR spectra of our extensive series of *N*-unsubstituted and *N*-substituted BTHIQs indicated that the preference observed in simple analogues for extended or folded conformations, respectively, is quite general, even when C-7 bears a bulky benzyloxy group.

The ^{1}H NMR data of all the compounds lacking an *N*-substituent (**1a**, **3a**, **4a**, **5a**, **6a**, **7a**, **8a**, **9a**) showed that, as in simpler BTHIQ derivatives,[16,18] an "extended" conformation (with the 1-benzyl moiety pointing away from the isoquinoline benzene ring) predominates. On the other hand, substitution at the nitrogen atom with methyl, ethyl, or propyl groups led to progressively increased shielding of H-8, of the C-7 methoxy protons, or of the C-7 benzyloxy methylene protons as in compounds **6b**−**d**, indicating that the *N*-substituted molecules reside most of the time in a "folded" conformation. Interestingly, in these molecules the C-7 benzyloxy methylene protons appeared as two distinct doublets, showing that this group is conformationally fixed on the NMR time scale. The *N*-substituted compounds studied here are therefore not only structural but also conformational mimics of the constrained "eastern" half of the tetrandrine molecule. As will be discussed later, the conformational preferences of these coclaurine derivatives may be related to their affinities at the prazosin binding site of the α_1-adrenoceptor and at the benzothiazepine site of L-type calcium channels.

The specific binding of the α_1-adrenergic ligand [^{3}H]-prazosin at a concentration of 0.2 nM and [^{3}H]diltiazem at a concentration of 3 nM represented approximately 90% and 70% of the total binding, respectively. All the compounds competed for both radioligands with the inhibition constants summarized in Table 2. The interactions of some representative coclaurine analogues with [^{3}H]prazosin- and [^{3}H]diltiazem-labeled rat brain cortical membranes are shown in Figures 1A and 1B. The low micromolar affinity of coclaurine (**1a**) for prazosin binding sites, which is not significantly different from that of tetrandrine, is slightly better than those of most of its analogues tested here.

On going from coclaurine to its *N*-methyl and *N*-ethyl derivatives (**1b** and **1c**), affinity for prazosin binding sites was halved with each additional carbon atom. This loss of affinity might be related to steric hindrance of a key interaction between the nitrogen atom, which should be largely protonated at physiological pH, and an anionic site common to all monoamine G-protein-coupled receptors. An alternative explanation could be that since an "extended" conformation is clearly less accessible in the *N*-substituted molecules, this less populated form may approximate the pharmacophoric conformation of BTHIQs at α_1-adrenoceptors. However, since flexible molecules may be biologically active without necessarily preferring a pharmacophoric conformation, but merely being able to adopt it when

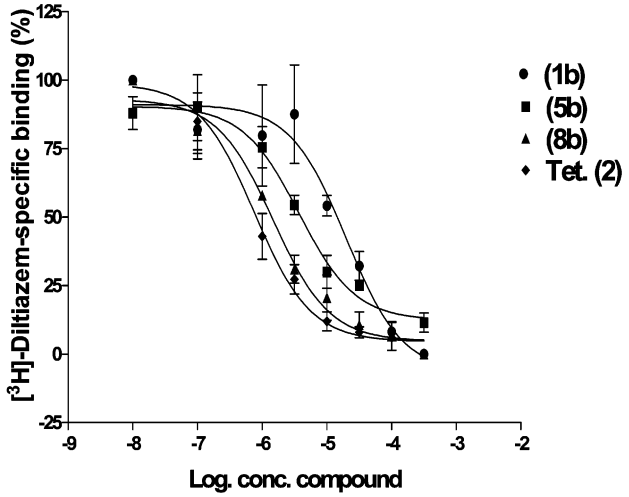

Figure 1. Displacement of the specific binding of [^{3}H]prazosin (A) or [^{3}H]diltiazem (B) (concentration−response curves) to rat cerebral cortical membranes by coclaurine, tetrandrine, and some derivatives. Each point is the mean of the results from three or five experiments performed in duplicate.

interacting with their macromolecular target, these results should be interpreted with caution. A similar, though less pronounced trend was seen in the more weakly binding 7-*O*-methylated norarmepavine series (**3a**−**c**) but not for 12-*O*-methylcoclaurine (**4a**) and its *N*-methyl analogue (**4b**), suggesting that a free hydroxyl group at C-12 might be a key feature of BTHIQ binding to α_1-adrenoceptors. This idea seems to be borne out by the low affinities of the 7,12-di-*O*-benzylated series (**6a**−**d**). The 7-*O*-benzyl compounds tested with a free 12-hydroxyl group (**7a**, **7b**) have higher affinities than coclaurine. 7-*O*-Benzyl-12-*O*-methylcoclaurine also (**8a**) bound somewhat better, but this gain was lost upon *N*-methylation. Finally, the affinity of 12-*O*-benzylcoclaurine (**9a**) was indistinguishable from that of the parent compound, possibly reflecting a different binding mode.

Micromolar coclaurine (**1a**) was unable to displace [^{3}H]-diltiazem from its brain cortical binding sites by more than 30%, while tetrandrine was a fairly potent displacer. In the present study we found that the *N*-substituted and *O*-methylated derivatives of coclaurine (**1b**,**c**, **3b**,**c, 4a**,**b**, **5b**) were able to displace the radioligand completely, although their affinities for the diltiazem binding site are only in the 10^{-5} M range. On the other hand, the *O*-benzylated derivatives tested had uniformly higher affinities than any of the other compounds, suggesting that large lipophilic substituents at C-7 and/or C-12 of the BTHIQ skeleton favor binding to the diltiazem site. Significantly, these are the positions occupied by the benzene rings of one of the halves of tetrandrine. When a direct comparison can be made between *N*-unsubstituted and *N*-substituted analogues, no definite conclusion can be drawn regarding the conformational preferences of these compounds at the diltiazem site, since their affinities do not show any consistent variation. In this regard, a recent molecular modeling study suggests that BTHIQs may be superimposed upon the diltiazem structure in an extended conformation and that this also appears to be the situation for the "western" half of tetrandrine.[21] All four mono-*O*-benzylated derivatives tested by us were statistically indistinguishable from tetrandrine as [^{3}H]diltiazem displacers, and as they are racemic compounds, the affinities of their eutomers might be expected to be greater by a factor of 2.

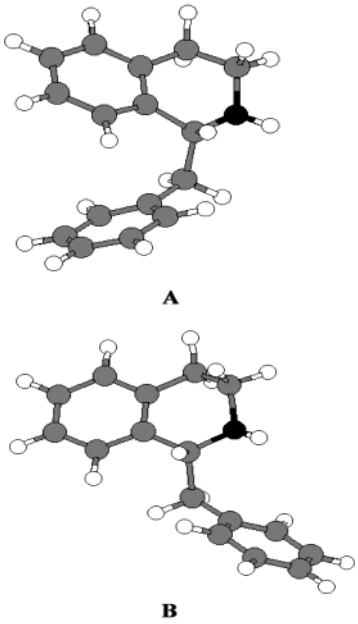

Figure 2. Semifolded (A) and extended (B) conformers of benzyltetrahydroisoquinoline, conformations optimized at the ab initio RHF-631g [d,p] level.

The IC_{50} values obtained for the different coclaurine analogues from functional studies in rat thoracic aortal rings are shown in Table 3. These results extend an earlier study showing that both coclaurine (**1a**) and norarmepavine (**3a**) relax this tissue, suggesting that these alkaloids are calcium channel blockers.[22] In our hands, cumulative increments of the concentrations of these compounds (10^{-9}−10^{-4} M) relaxed both noradrenaline (100 μM)- and KCl (80 mM)-induced contractions in the presence of Ca^{2+}, confirming their antagonism at adrenoceptors and calcium channels.

In these studies it is noteworthy that, in addition to being more potent adrenolytics, the 7-*O*-benzyl derivatives **7a** and **7b** are more potent than tetrandrine as blockers of KCl-elicited aortal contraction, and all four *O*-benzylated derivatives tested are more effective than coclaurine or tetrandrine as relaxants of vascular smooth muscle contracted with noradrenaline. Although tetrandrine has slightly higher affinities than these four compounds for the

Table 3. Inhibitory Potencies (pIC_{50}) of Coclaurine, Some Coclaurine Derivatives, and Tetrandrine on Contractions Induced in Rat Thoracic Aorta by Noradrenaline (1 μM) or KCl (80 mM)[a]

compound	NA pIC_{50}	KCl pIC_{50}
1a	4.76 ± 0.09	(17.2%)
1b	4.61 ± 0.14	4.17 ± 0.20
3b	3.68 ± 0.13	3.94 ± 0.07
4b	4.60 ± 0.22	4.14 ± 0.06
5b	4.18 ± 0.13	4.83 ± 0.07
7a	4.88 ± 0.10	5.12 ± 0.07
7b	5.06 ± 0.21	5.24 ± 0.16
8b	5.37 ± 0.12	4.94 ± 0.15
9a	4.79 ± 0.07	4.86 ± 0.07
tetrandrine (**2**)[15]	4.53	4.87

[a] Data are expressed as mean ± SEM; all binding experiments were repeated 4−6 times. Number in parentheses indicates % relaxation of contraction at 100 μM drug concentration.

[^{3}H]prazosin and [^{3}H]diltiazem binding sites, its lower functional potency, particularly at α_1-adrenoceptors, might result from different receptor subtype and ion channel populations in brain cortex and aorta or might be a consequence of its excessive molecular weight (623), limiting its access to its sites of action.

Regarding the effect of *N*-substitution, the α_1-adrenoceptor affinity of coclaurine (**1a**) and its *O*-methylated analogues (**3a** and **4a**) tends to fall off with increasing size of the *N*-alkyl groups in the *N*-alkylated compounds **1c** and **3c**. Two alternative but not exclusive interpretations may be put forth: either bulky substituents interfere with the presumably ionic interaction of the protonated nitrogen atom with an anionic residue, or they destabilize an extended conformation necessary for optimal binding to the receptor.

The affinity of **1a** and its *O*-methylated analogues (**3a** and **4a**) for α_1 receptors decreases with increasing methylation, suggesting that at least one of the hydroxyl groups may contribute to binding to the prazosin site. On the other hand, a benzyl ether moiety on the coclaurine structure allows affinity for this site to be preserved, even if the remaining hydroxyl group is methylated, suggesting that the additional benzyl group may establish hydrophobic interactions with relatively distant residues, compensating for the loss of the putative hydrogen bond donating hydroxyl groups. It must be kept in mind that different drugs competing for a common binding site may bind in different orientations. This goes to say that different pharmacophoric conformations of α_1-adrenolytic BTHIQs are likely to exist, in line with the wide structural variations among α_1-adrenergic antagonists.

The results obtained in the [^{3}H]diltiazem displacement experiments were quite different and practically independent of the presence of free phenolic functions. In this set of experiments, a single *O*-benzyl substituent, either at C-7 or C-12 (as in **7b, 8a**,**b**, and **9a**), or two *O*-benzyl groups (**6b** and **6d**) seem to favor binding to the benzothiazepine site over all the simpler BTHIQs. Blocking the free NH or OH with methyl groups also seems to increase the affinity of these substances, suggesting that the diltiazem binding site is rather hydrophobic. The increase in affinity upon *N*-substitution of **6a** to **6b**,**c** might also be taken as an indication that a "folded" conformation is preferred, but this interpretation clashes with the modeling studies cited above.[21] The functional responses of α_1-adrenoceptors and calcium channels to *O*-methylated coclaurine derivatives parallel their affinities for these compounds. Taken together, these results support our hypothesis that large, lipophilic substituents attached to the phenol groups of the coclaurine molecule are able to mimic the binding and functional interactions of tetrandrine with the prazosin and benzothiazepine sites in α_1-adrenoceptors and L-type calcium channels. Thus, extension of the coclaurine structure (which acts predominantly via antagonism at α_1-adrenoceptors) with *O*-benzyl substituents leads to enhanced smooth muscle relaxant potency involving both α_1-adrenergic antagonism and calcium channel blockage in the same narrow concentration range, suggesting the possibility of developing useful hypotensive drugs with a dual mechanism of action based on the BTHIQ scaffold.

Experimental Section

Some of the compounds reported here are known intermediates in the synthesis of natural BTHIQs. Most of them, however, have not been described previously, and their physical, spectroscopic, and analytical data are given as Supporting Information. Descriptions of the binding and functional studies are also included in the Supporting Information.

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Supporting Information Available: Experimental Section and descriptions of the binding and functional studies. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Schiff, P. L., Jr. *J. Nat. Prod.* **1991**, *60*, 934−953.
(2) Buck, K. T. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1987; Vol. 30, pp 1−222.
(3) Kulka, M. Bisbenzylisoquinolines. In *The Alkaloids*; Manske, R. H. F., Holmes, H., Eds.; Academic Press: New York, 1954; Vol. 4, pp 199−243.
(4) D'Ocon, P.; Blázquez, M. A.; Bermejo, A.; Anselmi, E. *J. Pharm. Pharmacol.* **1992**, *44*, 579−582.
(5) Felix, J. P.; King, J. F.; Shevell, J. L.; García, M. L.; Kaczorowski, G. J.; Bick, I. R. C.; Slaughter, R. S. *Biochemistry* **1992**, *31*, 11780−117893.
(6) Zafra-Polo, M. C.; Tormos, M. J.; Cortes, D.; Anselmi, E. *J. Pharm. Pharmacol.* **1993**, *45*, 563−566.
(7) Anselmi, E.; Gómez-Lobo, M. D.; Blázquez, M. A.; Zafra-Polo, M. C.; D'Ocón, M. P. *Pharmazie* **1994**, *49*, 440−443.
(8) King, V. F.; García, M. L.; Himmel, D.; Reuben, J. P.; Lam, Y. T.; Pan, J.; Han, G.; Kaczorowski, G. J. *J. Biol. Chem.* **1988**, *263*, 2238−2244.
(9) Wang, G.; Lemos, J. R. *Eur. J. Physiol.* **1992**, *421*, 558−595.
(10) Catret, M.; Anselmi, E.; Ivorra, M. D.; Elorriaga, M.; Tur, R.; D'Ocón, M. P. *J. Pharm. Pharmacol.* **1998**, *50*, 1267−1273.
(11) Kametani, T.; Yagi, H. *Chem. Pharm. Bull.* **1967**, *9*, 1283−1286.
(12) Kametani, T.; Takano, S.; Iida, H.; Shimbo, M. *J. Chem. Soc. C* **1969**, 298−300.
(13) Teitel, S.; Brossi, A. *J. Heterocycl. Chem.* **1968**, *5*, 825−829.
(14) Cava, M. P.; Buck, K. T. *Tetrahedron* **1969**, *25*, 2795−2805.
(15) Hori, H.; Nishida, Y.; Ohrui, H.; Meguro, H. *J. Org. Chem.* **1989**, *54*, 1346−1353.
(16) Dalton, D. R.; Cava, M. P.; Buck, K. T. *Tetrahedron Lett.* **1965**, *31*, 2687−2690.
(17) Marek, R.; Marek, J.; Dostal, J.; Slavík, J. *Collect. Czech. Chem. Commun.* **1997**, *62*, 1623−1630.
(18) Iturriaga-Vásquez, P.; Zapata-Torres, G.; Rezende, M. C.; Cassels, B. K. Unpublished results.
(19) Koike, L.; Marsaioli, A. J.; Rúveda, E. A.; Reis, F. *Tetrahedron Lett.* **1979**, *39*, 3765−3768.
(20) Schaeffer, P.; Lugnier, C.; Stoclet, J. C. *J. Cardiovasc. Pharmacol.* **1988**, *12* (Suppl. 4), S102− S103.
(21) Schleifer, K.-J.; Tot, E. *J. Comput.-Aided Mol. Des.* **2000**, *14*, 427−433.
(22) Morales, M. A.; Bustamante, S. E.; Brito, G.; Paz, D.; Cassels, B. K. *Phytother. Res.* **1998**, *12*, 103−109.

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