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Two-carbon bridge substituted cocaines: enantioselective synthesis, attribution of the absolute configuration and biological activity of novel 6- and 7-methoxylated cocaines

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Abstract

In an effort to learn more about the general structure–activity relationships of cocaine with the aim to elucidate those structural features that might confer antagonistic properties to such analogues, we describe herein our synthetic efforts to prepare two-carbon bridge functionalized (methoxylated and hydroxylated) analogues. Our approach makes use of a modification of the classical Willstatter synthesis of cocaine: Mannich type cyclization of acetonedicarboxylic acid monomethyl ester with methylamine hydrochloride and 2-methoxysuccindialdehyde in a citrate buffer solution afforded the 6- and 7-substituted 2-carbomethoxy-3-tropinones **3a**,**b** and **4a**,**b** in approximate yields of 64%. Reduction of the (±)-tropinone derivatives was performed with sodium amalgam in a sulfuric acid solution to afford a mixture of (±)-methoxyecgonine and (±)-methoxypseudoecgonine derivatives **5**, **11** and **6**, **7**, **12**, **13**. Benzoylation of these alcohols yielded the desired cocaine and pseudococaine-like compounds **8**, **14** and **9**, **10**, **15**, **16**. Additionally, we show that enzymatic hydrolysis of these cocaine analogues using pig liver esterase (PLE) affords a practical means for achieving their chemical resolution. The enantiomers of the methoxycocaine analogues were also prepared starting from chiral (+)- and (−)-6-methoxytropinone. All new analogues were examined for their ability to displace [^{3}H]mazindol binding and to inhibit high-affinity uptake of [^{3}H]dopamine into striatal nerve ending (synaptosomes). It appeared evident that methoxylation of the cocaine two-carbon bridge provides compounds of particular interest: the K_i for the binding of the methoxypseudococaines is about two to four times smaller than the K_i for inhibition of dopamine uptake, thus enabling these compounds capable of countering the effects of cocaine to some extent.

Keywords: Cocaine; Pseudococaine; Methoxycocaine derivatives; Antagonist properties; Enantioselective synthesis

1. Introduction

Cocaine abuse is one of the greatest concerns of society today and, in the US alone, one to three million cocaine abusers are estimated to be in need of treatment, six times the number of heroin addicts [1–3]. It is well documented that long-term cocaine abuse produces neurophysiological alterations in specific systems, in the central nervous system, that regulate the capacity to experience pleasure. Clinical and preclinical data about long-term cocaine exposure suggest cocaine causes a neurophysiological addiction [4].

Cocaine inhibits reuptake of the neurotransmitters norepinephrine and serotonin, as well as of dopamine. It binds to the dopamine transporter labelled by mazindol and to imipramine binding sites on serotoninergic neurons. Cocaine also affects neurotransmission in histamine, acetylcholine, and phenethylamine pathways. None of these neurotransmitter actions are solely responsible for cocaine euphoria since each action is also produced by other pharmacological agents that do not produce euphoria, are not self-administered by animals, and are not abused by humans [5–11]. Thus, although the rewarding properties of cocaine clearly require activation of dopaminergic systems, the molecular mechanism involved and whether activation is due only to

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direct cocaine effects on dopaminergic neurons, or to simultaneous collateral actions on other neurotransmitters, is uncertain.

Several laboratories have shown that cocaine inhibition of dopamine uptake into striatal synaptosomes is consistent with a classic, fully competitive mechanism [12–14]. However, these data are also consistent with more complex models, including allosteric or partially competitive models and several others involving steric hindrance, distinct but overlapping binding sites, or multiple binding sites in which at least one site is required for both cocaine and dopamine binding [15,16].

Present evidence has demonstrated that there are at least two binding sites for (*R*)-cocaine associated with the dopamine transporter, one with high and another with low affinity [17].

Recently, it has been demonstrated that cocaine, DA and amphetamine afford different protection against *N*-ethylmaleimide (NEM) alkylation of [^{3}H]mazindol binding sites: while cocaine, DA and amphetamine can protect the most reactive site from NEM alkylation, only cocaine can protect the less reactive site [18]. This would appear to allow both classically competitive and allosteric mechanisms for the inhibition of DA uptake by cocaine. Furthermore, it has been demonstrated that two aminoacidic residues, namely aspartate and serine, are crucial for dopamine transporters and have different importance for cocaine binding and dopamine uptake [19].

Taken together, these results support the notion that cocaine occupies a site that has both a distinct domain as well as a domain that it shares with other substrates such as DA and D-amphetamine. Consequently, this distinction may be sufficient to allow appropriately designed drugs which bind to a region of the cocaine binding domain without inhibiting dopamine uptake.

Table 1
Binding and dopamine uptake data for racemic compounds **8**, **9**, **14**–**16**

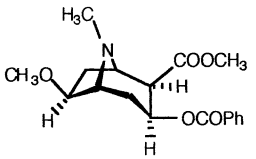
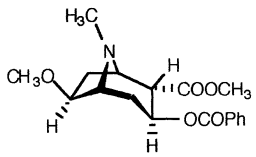
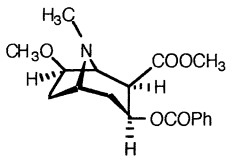
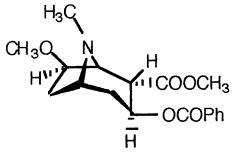
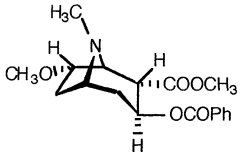
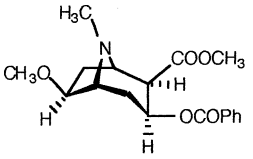
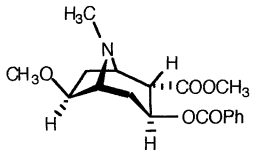
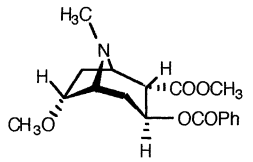
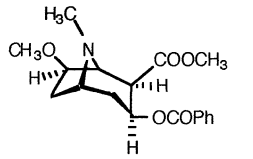
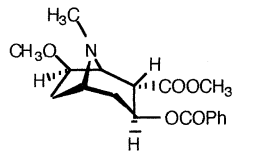
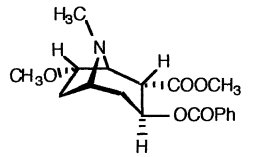
Compound		[^{3}H]Dopamine uptake K_i (μM)	[^{3}H]Mazindol binding K_i (μM)
Cocaine		0.32 ±0.01	0.28 ± 0.06
	(±)-**8**	68 ± 5	98 ± 12
	(±)-**9**	510 ± 110	190 ± 11
	(±)-**14**	6.1 ± 0.2	4.2 ± 0.1
	(±)-**15**	110 ± 4	45 ± 5
	(±)-**16**	200 ± 70	54 ± 3

Table 2
Binding and dopamine uptake data for enantiomers **8–10** and **14–16**

Compound		[^{3}H]Dopamine uptake K_i (μM)	[^{3}H]Mazindol binding K_i (μM)
Cocaine		0.32 ±0.01	0.28 ± 0.06
Pseudococaine		13.8 ± 1.5	10.4 ± 0.3
	(±)-**8**	68 ± 5*	98 ± 12*
	(-)-**8**	40.1 ± 8.1	38.4 ± 5.5
	(±)-**9**	510 ± 110*	190 ± 11*
	(+)-**9**	346 ± 66[nc]	113 ± 14
	(-)-**9**	313 ± 65[nc]	125 ± 12[nc]
	(±)-**10**	> 1,000[nc]	151 ± 13[nc]
	(±)-**14**	6.1 ± 0.2*	4.2 ± 0.1*
	(±)-**15**	110 ± 4*	45 ± 5*
	(+)-**15**	436 ± 34[nc]	133 ± 15[nc]
	(-)-**15**	44.6 ± 0.94	19.8 ± 6.4
	(±)-**16**	200 ± 70*	54 ± 3*
	(+)-**16**	325 ± 18[nc]	125 ± 6.6[nc]
	(-)-**16**	86.4 ± 1.32	59.9 ± 7.9

[a] Data of racemates previously reported [29].
[b] Less than 85% inhibition at highest concentration tested, 1,000 mM.

Based on this supposition, we hypothesized that modifications of the basic skeleton of the tropane ring of cocaine could afford compounds that allosterically diminish cocaine binding, but with cocaine antagonist properties. Support for a cocaine-based chemical approach can be found in the opiate area where useful narcotic antagonists have been discovered through simple modifications of morphine [20]. Furthermore, we were encouraged in this direction by the observation that substitution at the nitrogen portion of cocaine provides compounds endowed with cocaine antagonist activity [21].

On the basis of a large body of SAR work available principally from Carroll and co-workers [17,22–33], we decided to explore the effect of substitution at the two-carbon bridge of cocaine. In particular we considered of interest to study new cocaine derivatives bearing a methoxy function at the two-carbon bridge. In contrast to 2,3,4-substituted tropanes, compounds with substituents in the 6- or 7-positions are sterically con-

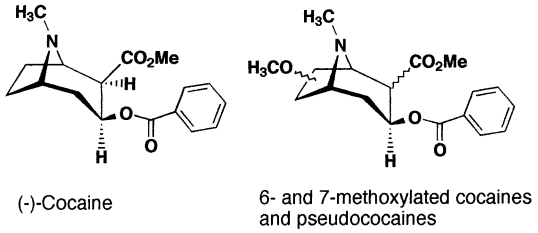
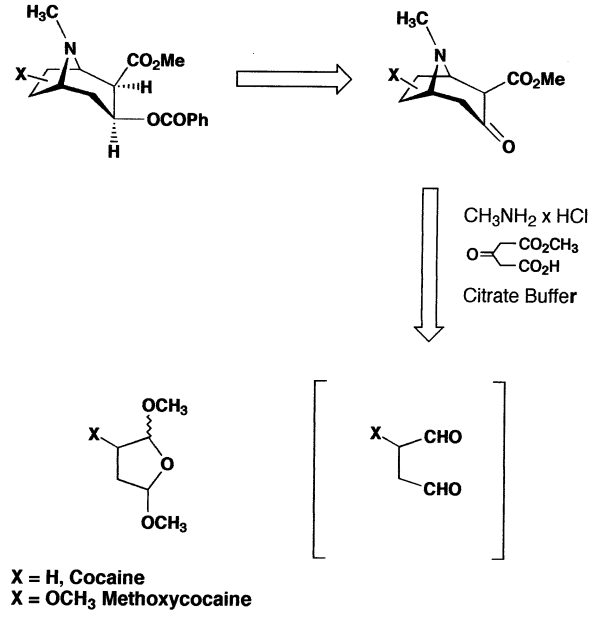


Fig. 1.

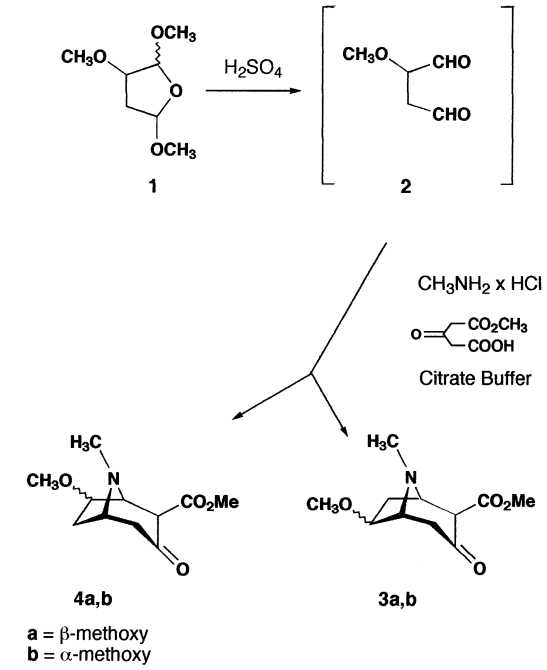
strained due a fixed spatial relationship between the tropane nitrogen and the above cited substituents. This could offer a potential tool for the study of pharmacophore–receptor interactions. Moreover, in our opinion, neglect of the two-carbon bridge substituted cocaines is a consequence of synthetic difficulties, principally in terms of the number of isomers which potentially could be obtained when a substituent is introduced in such positions.

Preliminary results from our laboratory (see Tables 1 and 2; Fig. 1) demonstrated that methoxylation of the cocaine two-carbon bridge provides compounds of particular interest: it appears that the K_i for the binding of methoxypseudococaines is about two to four times smaller than K_i for inhibition of dopamine uptake, thus making these compounds capable of countering the effects of cocaine to some extent [29].

Since it has been well documented by Carroll et al. that stereochemistry plays an important role in modulating the binding of cocaine analogues and that the seven other stereoisomers of cocaine are less potent than the original one [30,33], we have been developing a methodology aimed at optical resolution of our racemic mixtures.



Scheme 1.



Scheme 2.

In this lecture we describe the preparation of racemic 6- and 7-methoxylated cocaines **8** and **14** and the pseudococaine-like derivatives **9**, **10**, **15** and **16**, as well as the 6- and 7-hydroxylated analogues **28a**,**b** and **29a**,**b**, together with new findings emerged during their optical resolution. Biological results for racemates and enantiomers of the methoxylated cocaines will also be reported.

2. Synthesis of racemic methoxycocaines 8, 14 and pseudomethoxycocaines 9, 10, 15 and 16

Since (−)-cocaine is the usual starting material, synthetic flexibility is naturally restricted, causing serious limitations in the preparation of cocaine analogues: the logical consequence of this type of approach is the lack of information about the biology of cocaine stereoisomers. In order to overcome this limitation we reconsidered the chemistry developed nearly seven decades ago by Wilstatter et al. [34] (see retrosynthetic analysis in Scheme 1) as a potential tool in synthesizing cocaine-like derivatives as well as its stereoisomers: Mannich type cyclization of acetonedicarboxylic acid monomethyl ester with methylamine hydrochloride and succindialdehyde in a citrate buffer solution affords the 2-carbomethoxy-3-tropinone. Reduction of the latter tropinone intermediate may affords cocaine-, pseudococaine- and allopseudococaine-like derivatives.

Keeping this approach in mind, it was easy to go about the introduction of different substituent on the cocaine carbon skeleton ring starting from derivatives

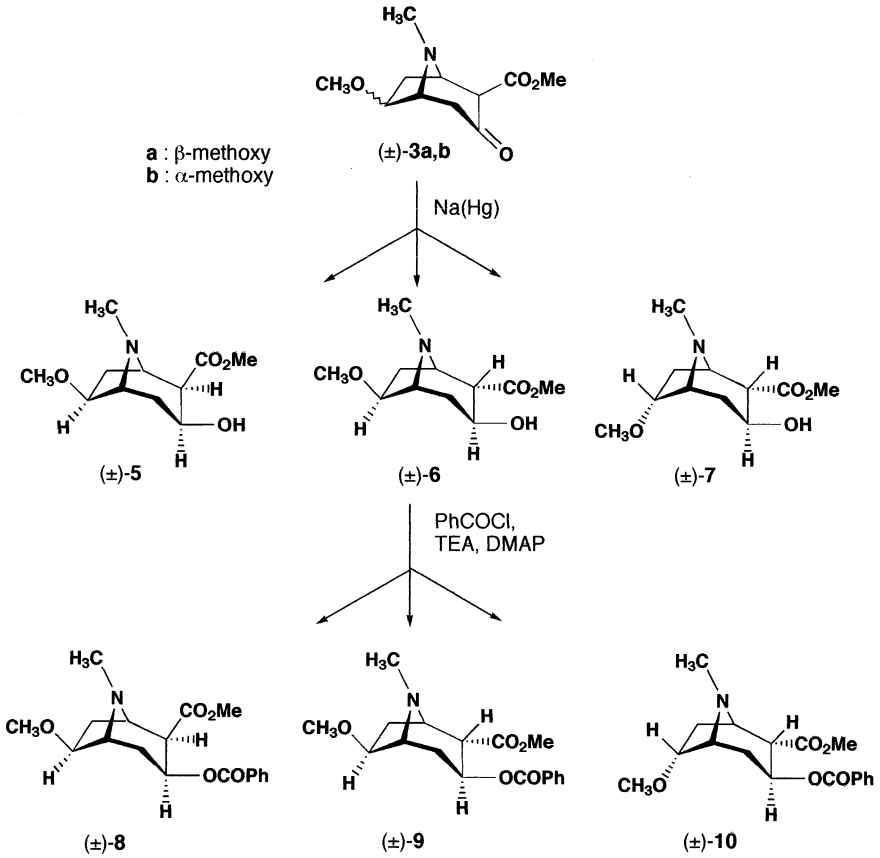
of acetonedicarboxylic acid monomethyl ester and succindialdehyde. Retrosynthetic analysis (Scheme 1) suggested that 6- or 7-methoxy-substituted 2-carbo-methoxytropinone derivatives **3a**,**b** and **4a**,**b** incorporating the methoxy function present in our desired cocaine derivatives **8**–**10** and **14**–**16** could be suitable candidates.

Accordingly, the desired two-carbon bridge methoxycocaine derivatives **8**–**10** were prepared as reported in Scheme 3, while the 2-carbomethoxytropinone derivatives **3a**,**b** and **4a**,**b** were obtained starting from the known methoxysuccindialdehyde (**2**) (Scheme 2). Finally, compounds **14**–**16** were prepared in the same manner as described for **8**–**10** starting from **4a**,**b** (Scheme 4).

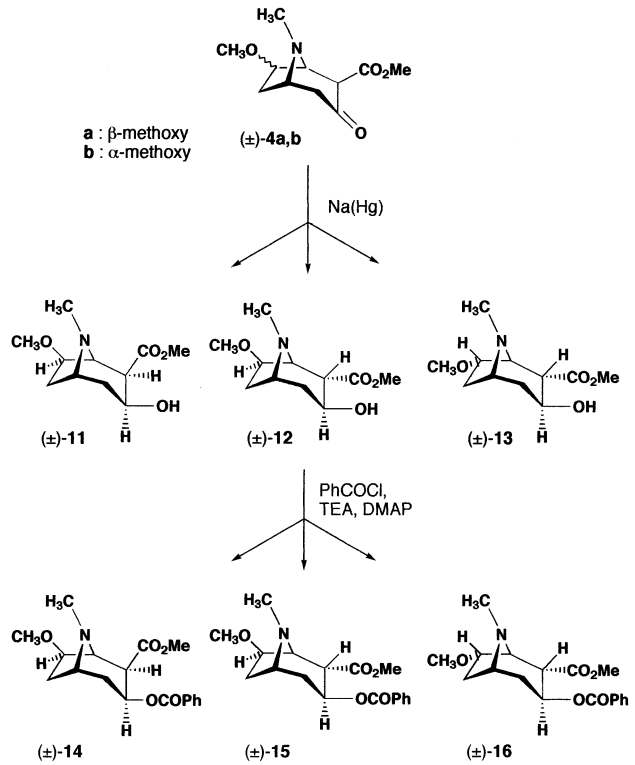
The monomethyl ester of acetonedicarboxylic acid, prepared from the acetonedicarboxylic acid anhydride and methanol at room temperature [35–37], was reacted with methylamine hydrochloride and 2-methoxysuccindialdehyde (**2**) in a citrate buffer solution to give the key tropinone derivatives **3a**,**b** and **4a**,**b** in 64% yield (Scheme 2). The reaction produced a mixture (1:1 ratio) of the (±)-6β-methoxy-2-carbomethoxy-3-tropinone (**3a**) together with traces of the 6α isomer **3b** and the (±)-7β-methoxy-2-carbomethoxy-3-tropinone (**4a**). Only a limited amount (6%) of the (±)-7α-methoxy-2-carbomethoxy-3-tropinone (**4b**) was recovered. The starting material 2-methoxysuccindialdehyde (**2**) was obtained by exposure of a mixture of 2,3,5-trimethoxytetrahydrofuran (**1**) to a 0.2 M aqueous sulfuric acid solution. The 2,5-dimethoxy-2,5-dihydrofuran was reacted with methanol [38] to afford the desired 2,3,5-trimethoxytetrahydrofuran (**1**). Reduction of the (±)-methoxytropinone derivatives **3a**,**b** and **4a**,**b** was performed with sodium amalgam [35], in a pH 3–4 sulfuric acid solution, to give a mixture of the (±)-methoxyecgonine derivatives **5**, **11** and the (±)-methoxypseudoecgonine derivatives **6**, **7**, **12**, **13**. All compounds were purified by flash chromatography, the alcohol (±)-**7** was obtained as an inseparable mixture with (±)-**6** and used in the next step without any further purification.

Benzoylation of alcohols **5**–**7** and **11**–**13** using benzoyl chloride in the presence of triethylamine (TEA) and a catalytic amount of 4-dimethylaminopyridine (DMAP) produced the desired cocaine- and pseudococaine-like derivatives **8**–**10** and **14**–**16** in 53–71% yield.

Structural assignments of the newly prepared benzoyl derivatives were inferred by ^{1}H and ^{13}C NMR analyses and by comparison with data of literature [39]. Positions of all protons in the tropane ring were assigned on the basis of proton-decoupling experiments, starting



Scheme 3.

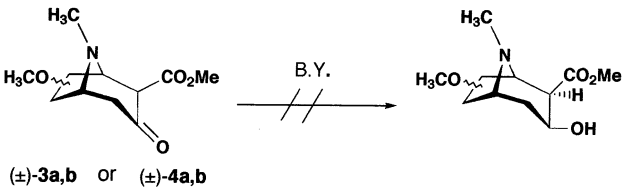


Scheme 4.

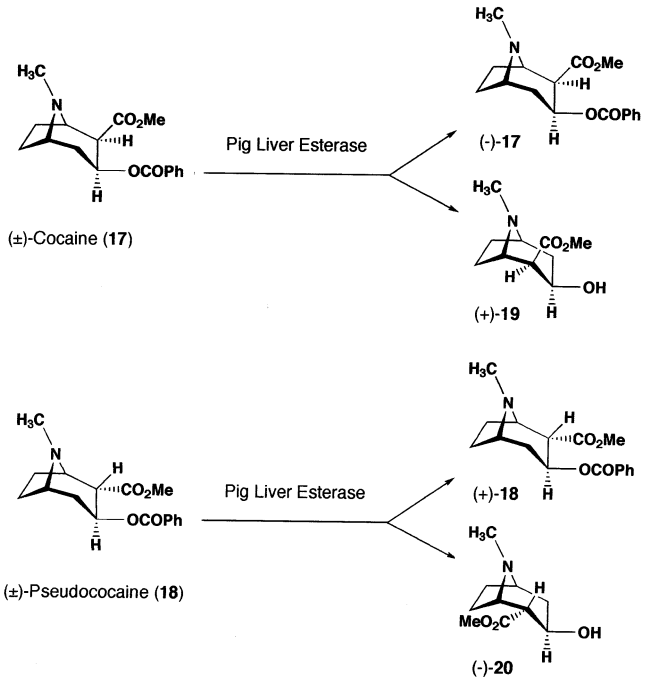
from the diagnostic H-3 proton that appears as a multiplet centered at 5.80 ppm. Consequently, the H-6 proton, linked to the methoxy function, was easily attributed in the 4.06 ppm region. The stereochemistry was attributed on the basis of the coupling constants between H-2 and H-3, H-5 and H-6 protons. A constant of about 6 Hz between H-5 and H-6 was indicative of an α-configuration of the methoxy function whereas 10.6 Hz between H-2 and H-3 protons indicated a pseudococaine-like structure. Finally, a constant of about 6 Hz between H-2 and H-3 was indicative of a cocaine-like configuration.

3. Pig liver esterase (PLE) resolution of racemic 6- and 7-methoxycocaines analogues 8–10 and 14–16

Initial attempts to resolve the racemic mixtures **8**–**10** and **14**–**16** by using the classical tartaric acid resolution, as described by Carroll et al. [30], were unsuccessful. Moreover, further attempts (Scheme 5) involving the baker's yeast (B.Y.) reduction of the carbomethoxy-tropinone analogues **3a**,**b** and **4a**,**b**, gave as unique result the formation of the corresponding 6-methoxy-3-tropinone. Although a large body of literature exists concerning the use of the β-ketoester moiety in microbial transformations aimed at the production of chiral building blocks, the present finding was surprising and, to the best of our knowledge, unprecedented.



Scheme 5.



Scheme 6.

Since the sodium-amalgam based reduction of the 2-carbomethoxy-3-tropinone is the only known access to cocaine-like compounds, the enantioselective reduction of compounds **3a**,**b** and **4a**,**b** did not appear readily feasible.

Recently, it was reported that cocaine is inactivated in vivo through a methabolic pathway which involves hydrolysis of the ester in position 3 [40,41]. This reaction affords the formation of the ecgonine methyl ester. Moreover, it has been well documented that (1*S*)-cocaine is faster hydrolyzed, by baboon plasma butyrylcholinesterase, than the corresponding (1*R*)-isomer [42].

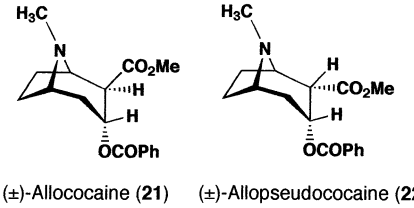
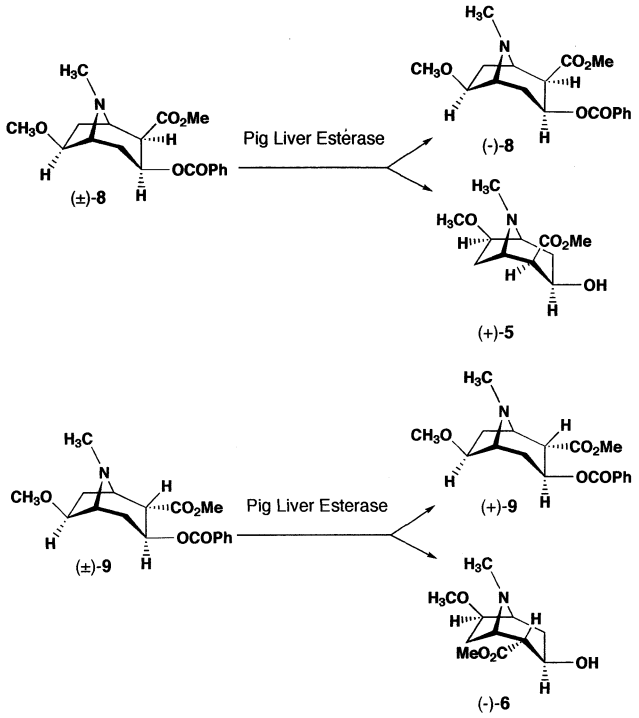


Fig. 2.



Scheme 7.

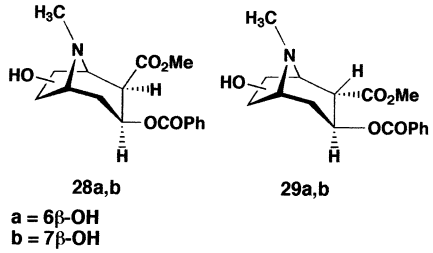


Fig. 3.

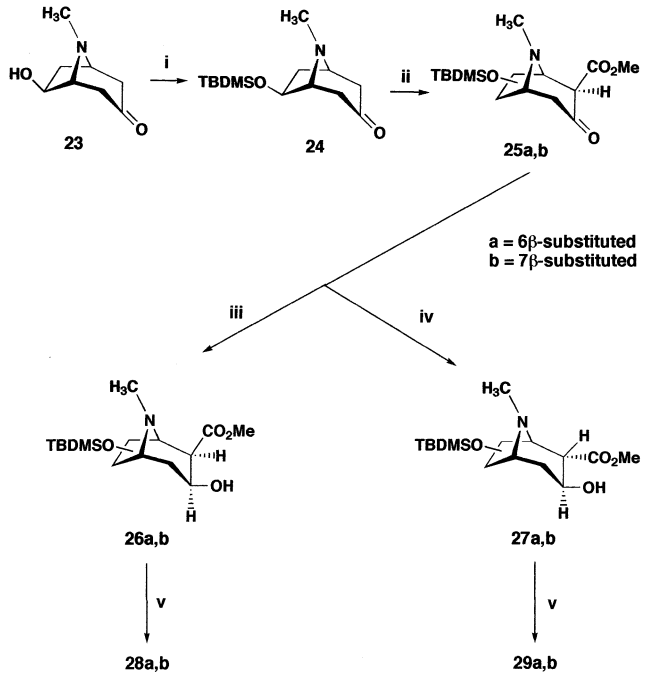
In view of these observations and of our own work in which some of us have shown that PLE has a particular affinity for the benzoic ester moiety [42], we were attracted to effect an enzymatic resolution of our racemic mixture.

The PLE-catalyzed hydrolysis of cocaine and pseudococaine (Scheme 6) was readily performed in aqueous solution at 37°C, maintaining the pH at 7. The hydrolysis was continued until one-half equivalent of sodium hydroxide had been consumed. The reaction time was about 1 h for pseudococaine, whereas the hydrolysis of the cocaine molecule required longer reaction times (7 h). In the case of (±)-pseudococaine, preferential hydrolysis of the (−)-isomer takes place to afford (−)-pseudoecgonine methyl ester (−)-**20**. In contrast, for cocaine the (+)-isomer is hydrolyzed at a faster time producing (+)-**19**. Attempts to effect the PLE-catalyzed hydrolysis of (±)-allococaine (**21**) and (±)-allopseudococaine (**22**) (Fig. 2) failed; the first analogue was recovered unchanged under the reaction conditions described, whereas the second compound was found to undergo slow decomposition. The more rapid rate of hydrolysis of (+)-cocaine by PLE is consistent with an earlier report of the preferential hydrolysis of (1*S*)-cocaine relative to its (1*R*)-counterpart which has been observed in baboon plasma [43]. The preferred hydrolysis of the equatorial –OCOPh group can be rationalized by considering the active-site model first proposed for PLE by the groups of Tamm [44] and Jones [45–47]. In the case of six-membered ring substrates, it has been shown that an equatorial, or pseudo-equatorial ester orientation is preferred by PLE.

With regard to the chemoselectivity of the hydrolysis, i.e. the selective cleavage of the benzoate group rather than the carbomethoxy group, we may advance the hypothesis that together with a higher susceptibility of the benzoate group to hydrolysis, the preferred enzyme–substrate complex must allow better alignment of the –OCOPh group with the serine residue present in the catalytic site. To this regard, it may be of interest to

Table 3
Products of PLE resolution

Starting material	Products	Yield (%)	$[\alpha]_D^{20}$	ee (%)
(±)-**17**	(−)-Cocaine	45	−13.1° (*c* 2.5, $CHCl_3$); −16° (*c* 4, $CHCl_3$) [26]	81.8
(±)-**17**	(+)-Ecgonine methyl ester hydrochloride	35	+41.5° (*c* 1.5, MeOH); +52.3° (*c* 1, MeOH) [13]	71
(±)-**18**	(+)-Pseudococaine hydrochloride	91	+43° (*c* 1, MeOH); +43° (*c* 1, MeOH) [27]	100
(±)-**18**	(−)-Pseudoecgonine methyl ester	85	−22.2° (*c* 2, H_2O); −22.5° (*c* 1, H_2O) [27]	95
(±)-**8**	(−)-6β-Methoxycocaine	35	−16.4° (*c* 1, MeOH)	82
(±)-**8**	(+)-6β-Methoxyecgonine methyl ester	3	+24.3° (*c* 2.3, MeOH)	95
	(+)-6β-Methoxycocaine	65	+16.1° (*c* 1, MeOH)	85
(±)-**15**	(+)-7β-Methoxypseudococaine	60	+35.9° (*c* 1, MeOH)	99
(±)-**15**	(−)-7β-Methoxypseudoecgonine methyl ester	30	−18.0° (*c* 3, MeOH)	99
	(−)-7β-Methoxypseudococaine	74	−37.0° (*c* 1, MeOH)	97
(±)-**16**	(+)-7α-Methoxypseudococaine	55	+46.1° (*c* 1, MeOH)	97
(±)-**16**	(−)-7-α-Methoxypseudoecgonine methyl ester	26	−14.2° (*c* 2.5, MeOH)	95
	(−)-7-α-Methoxypseudococaine	67	−46.7° (*c* 1, MeOH)	97



Scheme 8.

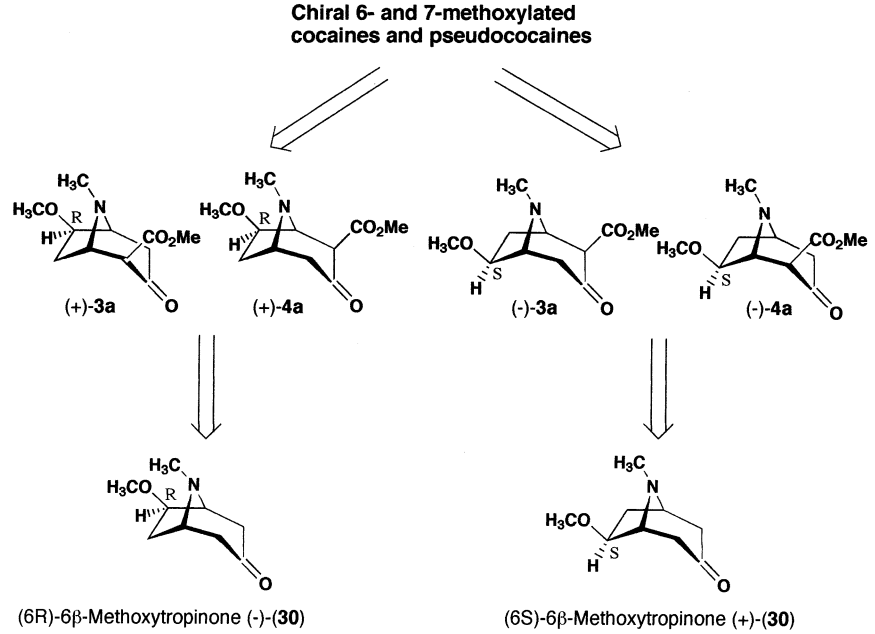
note that the use of longer reaction times, and consequently increased amounts of sodium hydroxide, led to the isolation of some ecgonine.

Very interestingly, we found that PLE works efficiently (Scheme 7) on our methoxycocaines affording a good separation of the two isomers. In the case of the pseudococaine (±)-**9**, the reaction was very fast and hydrolysis occurs preferentially for the (−)-isomer, affording the corresponding (−)-pseudoecgonine derivative (−)-**6**. This latter was, in turn, benzoylated leading to the final (−)-pseudococaine-like analogue (−)-**9**. On the contrary, the (+)-isomer was faster hydrolyzed, with respect to the (−)-isomer, in the case of the cocaine-like derivative (±)-**8**. This result is in agreement with the above reported on the faster hydrolysis of (1*S*)-cocaine compared to (1*R*)-cocaine.

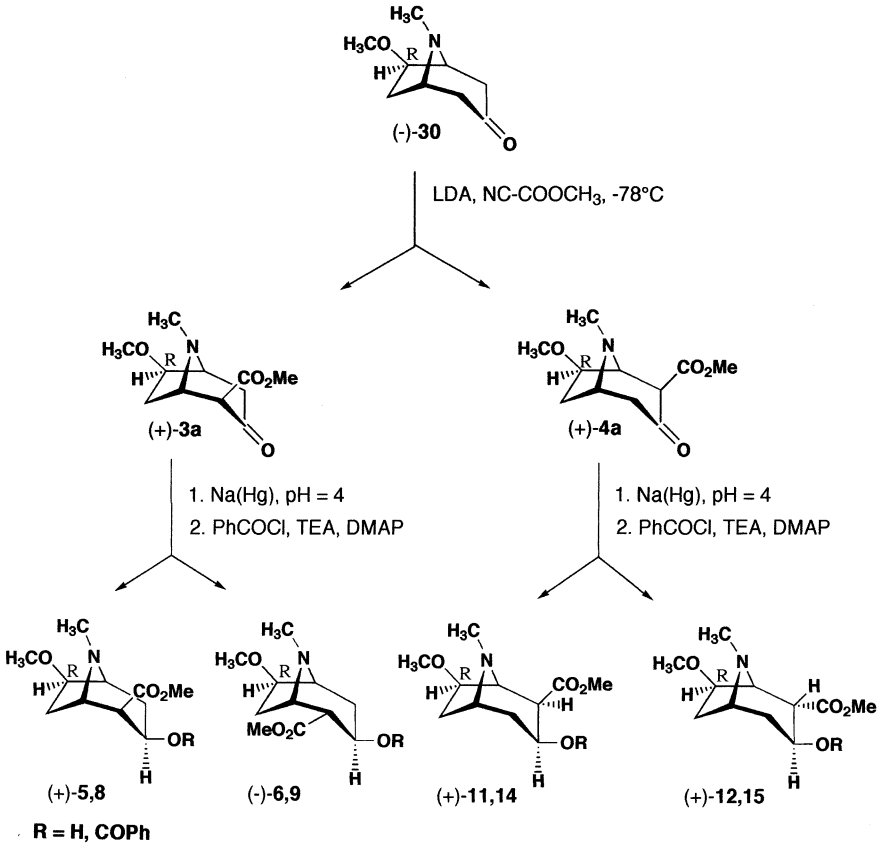
Therefore, application of the PLE-catalyzed hydrolysis reaction to the racemic mixture of cocaine and its analogues provides efficient access to the optically pure tropanes [48], all of which are obtained in good yields and enantiomeric excess (Table 3).

4. Synthesis of two-carbon bridge hydroxylated cocaines and pseudococaines 28a,b and 29a,b

To further explore the structure–activity relationships of cocaine we needed access to 6- and 7-hydroxylated cocaine analogues **28a**,**b**, as well as to their pseudo-stereoisomers **29a**,**b** (Fig. 3). In spite of the fact that tropane alkaloids bearing an oxygenated substituent at the two-carbon bridge are well represented in nature, a search of the literature failed to reveal any general methodologies for the synthesis of the above mentioned 6- and 7-hydroxylated cocaine derivatives. It appeared evident that ready access to the hydroxylated analogues **28a**,**b** and **29a**,**b** could be achieved by simple demethylation of the already-prepared methoxycocaines.



Scheme 9.



Scheme 10.

Unfortunately, all attempts to cleave the methyl ether group present in the starting methoxylated cocaines were unsuccessful because the ester function underwent cleavage more readily than the methyl group, thus producing inseparable mixtures of compounds. Consequently, we devised an alternative methodology starting from the 6-hydroxytropinone (**23**) (Scheme 8), which in turn was easily transformed into the β-ketoesters **25a**,**b**, two promising precursors of our desired hydroxycocaine derivatives **28a**,**b** and **29a**,**b**. Thus, the tropinone derivative **23** was prepared as described in literature and the hydroxy function was protected as its *t*-butyldimethylsilyl ether. The ketone was deprotonated with sodium hydride in the same manner as described by Carroll et al. for tropinone [39]. The enolate was reacted with dimethyl carbonate to afford in 85% yield a mixture (1:1) of the corresponding carbomethoxylated derivatives **25a**,**b**. Careful flash chromatography allowed the separation of the two isomers. Reduction of the β-ketoesters (with NaHg or $NaBH_4$) and subsequent benzoylation and desylilation allowed in good yields the desired racemic hydroxycocaine derivatives **28a**,**b** and hydroxypseudococaine derivatives **29a**,**b**. Therefore, this methodology provides ready access to the 6- and 7-hydroxylated derivatives of cocaine and pseudococaine [49].

5. Enantioselective synthesis and attribution of the absolute configuration of two-carbon bridge methoxylated cocaines

A search of literature revealed that the absolute stereochemistry of the majority of chiral natural tropane alkaloids is known in only a few cases [50–52]: the optical properties of the isolated compounds are usually not known and most of the syntheses of these natural products afford racemic mixtures. Therefore, the development of new methodologies for the enantioselective preparation of cocaine and its analogues still represents an attractive goal. Accordingly, we have devised a novel enantioselective approach for the preparation of two-carbon bridge methoxylated cocaines which in turn allows concomitant, facile attribution of their absolute configuration.

Retrosynthetic analysis suggested (Scheme 9) that chiral (6*R*)- and (6*S*)-2-carbomethoxytropinone derivatives (±)-**3a**, and the corresponding regioisomers (±)-**4a**, possessing the known configuration at the carbon atoms bearing the methoxy functionality, would constitute appropriate starting materials for our purpose.

It was anticipated that carbomethoxylation of the known chiral 6-methoxytropinones (±)-**30** would allow a facile access to the desired 2-carbomethoxy

derivatives (+)-**3a**, (+)-**4a**, (−)-**3a**, and (−)-**4a**. Moreover, their stereoselective reduction could afford the ecgonine methyl ester derivatives (+)-**5**, (+)-**11** and, of course, the corresponding enantiomers (−)-**5**, (−)-**11**, together with the pseudoecgonine derivatives (−)-**6**, (+)-**12**, (+)-**6** and (−)-**12**.

With this approach in mind, it was easy to imagine that absolute configuration of all the new synthesized methoxycocaines could be unambiguously assigned.

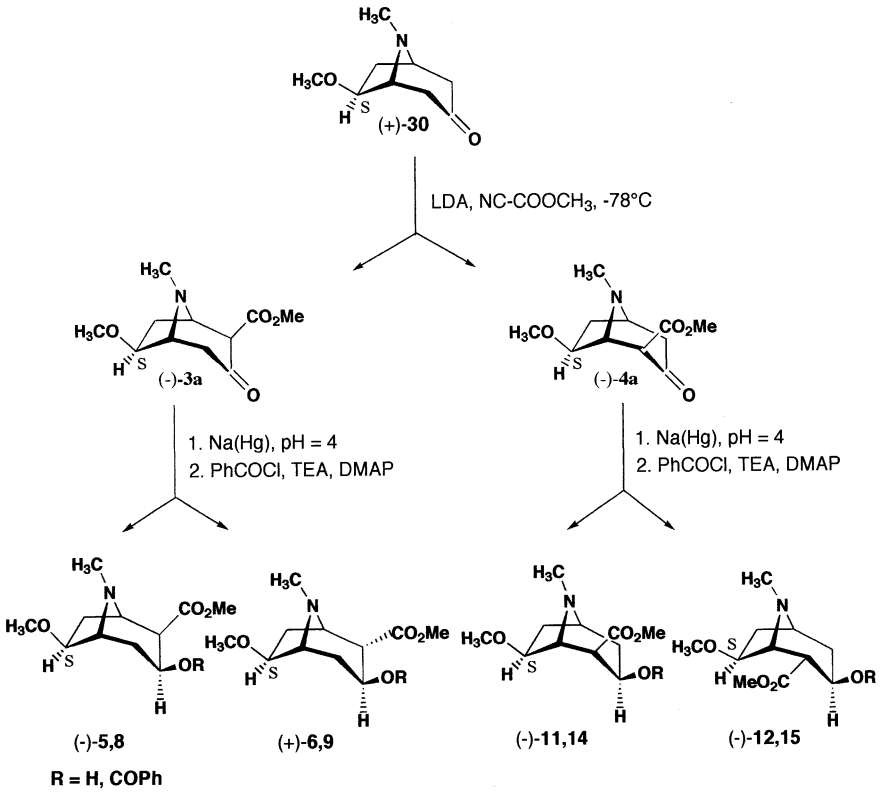
Thus, the carbomethoxylation of (−)-**30** (Scheme 10) afforded in 65% total yield the two regioisomers (+)-**3a** and (+)-**4a** bearing the carbomethoxy function at the 6- and 7-carbon atoms, respectively (see Scheme 11 for the carbomethoxylation of (+)-**30**). The β-ketoesters were reduced with sodium amalgam to generate both the ecgonine-like derivatives (+)-**5** and (+)-**11** and the pseudoecgonine-like derivatives (−)-**6** and (+)-**12** in 15–32 and 44–60% yields, respectively. Even though this transformation was not stereoselective, the two isomers were readily individualized by TLC and careful flash chromatography allowed them to be separated easily.

Benzoylation of (+)-**5**, (+)-**11**, (−)-**6** and (+)-**12** provided easily (+)-**8**, (+)-**14**, (−)-**9** and (+)-**15**. Interestingly, the (+)-(6β)-methoxytropinone (+)-**30**, afforded in the same manner as described above for the methoxytropinone (−)-**30**, the new (6*S*)- and (7*S*)-enantiomers (−)-**8**, (−)-**14**, (+)-**9** and (−)-**15**.

In conclusion, the use of the optical antipodes of the 6-β-methoxytropinone permits the synthesis of all the enantiomers (see ee in Table 4) of the 6-β- and 7-β-methoxycocaines as well as of the diastereoisomers 6-β- and 7-β-methoxypseudococaines. It is noteworthy that the synthetic methodology described above also allowed the assignment of absolute configuration of the new synthesized enantiomers.

6. Biological results and discussion

Enantiomers (−)-**8**, (+)-**9**, (−)-**9**, (+)-**15**, (−)-**15**, (+)-**16** and (−)-**16** and the racemic mixtures **10** and **14**, were examined for their ability to displace [^{3}H]mazindol binding, a compound which has been shown to label the cocaine binding sites on the dopamine transporter of rat striatal membranes. This ligand binds with high affinity to a single sodium-dependent site in striatal membranes, representing the dopamine carrier. Additionally, the new compounds were tested for their ability to inhibit high-affinity uptake of [^{3}H]dopamine into striatal nerve endings (synaptosomes). All synthesized compounds were less active as compared with the parent cocaine and pseudococaine. As a general rule, the (−)-isomers, belonging to both cocaine- and pseudococaine-like series, were more active than the racemic mixture in both, binding



Scheme 11.

Table 4
Synthetic chiral methoxy derivatives of cocaine and pseudococaine

Starting material	Products	Yield (%)	$[\alpha]_D^{20}$	ee (%)
(+)-**3a**	(+)-6β-Methoxyecgonine methyl ester (+)-**5**	15	+24.5° (*c* 1.5, MeOH)	91
(+)-**5**	(+)-6β-Methoxycocaine (+)-**8**	66	+18.3° (*c* 1.5, MeOH)	93
(+)-**4a**	(+)-7β-Methoxyecgonine methyl ester (+)-**11**	32	+50.5° (*c* 1, MeOH)	99
(+)-**11**	(+)-7β-Methoxycocaine (+)-**14**	75	+14.0° (*c* 2, MeOH)	99
(+)-**3a**	(−)-6-β-Methoxypseudoecgonine methyl ester (−)-**6**	44	−7.45° (*c* 1, MeOH)	97
(−)-**6**	(−)-6β-Methoxypseudococaine (−)-**9**	89	−30.3° (*c* 1.8, MeOH)	95
(+)-**4a**	(+)-7-β-Methoxypseudoecgonine methyl ester (+)-**12**	22	+39.5° (*c* 2, MeOH)	99
(+)-**12**	(+)-7β-Methoxypseudococaine (+)-**15**	85	+37.2° (*c* 2, MeOH)	99
(−)-**3a**	(−)-6β-Methoxyecgonine methyl ester (−)-**5**	19	−24.3° (*c* 1.5, MeOH)	94
(−)-**5**	(−)-6β-Methoxycocaine (−)-**8**	55	−18.0° (*c* 1, MeOH)	90
(−)-**4a**	(−)-7-β-Methoxyecgonine methyl ester (−)-**11**	25	−50.1° (*c* 1, MeOH)	97
(−)-**11**	(−)-7β-Methoxycocaine (−)-**14**	63	−14.0° (*c* 2, MeOH)	98
(−)-**3a**	(+)-6β-Methoxypseudoecgonine methyl ester (+)-**6**	60	+6.7° (*c* 1, MeOH)	95
(+)-**6**	(+)-6β-Methoxypseudococaine (+)-**9**	85	+29.3° (*c* 2, MeOH)	93
(−)-**4a**	(−)-7β-Methoxypseudoecgonine methyl ester (−)-**12**	44	−38.7° (*c* 1.5, MeOH)	99
(−)-**12**	(−)-7β-Methoxypseudococaine (−)-**15**	71	−37.0° (*c* 1.5, MeOH)	99

and DA uptake inhibition, biological tests. The (−)-**16** has the same degree of activity as compared to the racemic mixture: this data being in contrast with the expected pattern.

Important effects on activity were achieved by inversion of the configuration: a decrease of activity was observed for isomers (+)-**15** and (+)-**16** with respect to the corresponding (−)-isomers. On the contrary, compounds (±)-**9** showed a different activity profile as compared with the parent derivatives (±)-**15** and (±)-**16**: the two enantiomers were equally active and both showed an increased activity with respect to the racemic mixture.

Very interestingly, the K_i for the binding of compounds (+)-**9**, (+)-**15** and (+)-**16** is about 2.5–3 times smaller than their K_i for inhibition of dopamine uptake. As compared with the values reported previously for racemic mixtures, the differences are higher for derivatives (+)-**9** and (+)-**15**, but, very surprisingly, somewhat smaller for derivative (+)-**16**: this occurrence, in contrast to the expected pattern, is presently unexplained.

A difference between the binding affinities and dopamine reuptake inhibition is also retained for the parent (−)-pseudococaine-like derivatives (−)-**9** and (−)-**15** (K_i for binding is about 2.2–2.5 times smaller than their K_i for inhibition of dopamine uptake); on the contrary, the (−)-**8** and (−)-**16** isomers lose the discrepancy in binding versus dopamine reuptake inhibition.

Although the limited number of compounds does not allow us to draw conclusions, it appears that (+)-isomers could play an important role in the discovery of a cocaine antagonist.

Moreover, notwithstanding that the differences in K_i values for binding versus uptake inhibition are not dramatic, the results may still be significant. The data provided for these pseudococaine-like derivatives show that this difference does not relate solely to the α-stereochemistry of the carbomethoxy group: the position of the methoxy function in the two-carbon bridge as well as its stereochemistry seems to play an important role in modulating cocaine antagonist activity.

Therefore, these findings add further support to the idea that the cocaine recognition site may be distinct from the dopamine transporter: if cocaines were, in fact, simply binding within the transporter channel, then any analogue of cocaine would always inhibit dopamine uptake.

Since differential binding and dopamine uptake K_i values are obtained for compounds (+)-**9**, (+)-**15**, (+)-**16**, then the mazindol/cocaine binding site may in fact be located in a region which is distinct from the transporter channel. These compounds may therefore have the properties of partial agonists or antagonists, and thus be capable of countering to some extent the cocaine effects.

Finally, we believe of particular interest the biological results related to the new racemic 6-methoxypseudococaine derivative **10**: even though the introduction of a methoxy function at 6α-position seriously compromises binding affinity at the dopamine transporter ($K_i = 151 \pm 13$), the dramatic decrease of the capability to inhibit DA reuptake of [^{3}H]dopamine ($K_i > 1000$) into striatal nerve endings makes this compound of particular interest. Based on these latter findings, it appears reasonable to think that the introduction of a phenyl group in the 3-position ('Win' type analogue) could allow higher affinity at the dopamine transporter. Furthermore, it would be considered of interest to prepare additional compounds, related to **10**, in order to see whether further optimization of antagonist activity can be achieved.

7. Conclusions

The discrepancy observed in binding and dopamine uptake inhibition for the pseudococaine-like derivatives (+)-**9**, (+)-**15** and (+)-**13** together with the discovery of compound **10**, which exhibit a certain degree of affinity for the dopamine transporter but without evident inhibition of the dopamine uptake, make the idea of a cocaine-based approach in discovering a possible cocaine antagonist more robust. These data suggest that substitution at the two-carbon bridge of cocaine may afford derivatives which bind to the dopamine transporter without blockade and/or maintaining the normal dopamine uptake. This is the requisite for the discovery of a possible 'cocaine antagonist' clinically useful in the treatment of cocaine abusers.

Altogether, these data confirm the hypothesis that a significant portion of the cocaine binding domain on the dopamine transporter is distinct from that of either dopamine or amphetamine, and this distinction allows specifically designed drugs to prevent cocaine binding without inhibiting dopamine uptake.

In conclusion, notwithstanding that cocaine stereoisomer derivatives were scarcely considered until now, considerations emerging from our results provide evidence that stereoisomers may play an important role in the discovery of potential cocaine antagonists and are deserving of further research in this field.

Finally, about the PLE resolution of cocaine racemates, we believe that our results could support the hypothesis of a different activity of cocaine stereoisomers explained, at least in part, in terms of a different susceptibility to hydrolysis in plasma. To the best of our knowledge, the PLE resolution of cocaine derivatives represent a new entry for resolution of tropane-like racemic derivatives.

The elevated optical yields observed, the relatively simple experimental conditions required together with the possibility of working either very small or large scale preparation add a particular interest to this methodology, especially for researchers working in the cocaine field. This compares very favorably with alternative procedures and further extension of this methodology to the PLE resolution of racemic cocaine and its steroisomers is currently in progress.

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